EFFECTS OF ORGANOHALOGEN CONTAMINANTS ON STELLER SEA LION SURVIVAL AND FEMALE REPRODUCTION IN THE RUSSIAN FAR EAST

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Abstract

The presence of persistent organohalogen contaminants (OCs) in the habitats of Steller sea lions, *Eumetopias jubatus*, may influence reproductive rates and possibly survival. The lack of recovery and the reduction in natality for the western stock has no apparent cause and OCs may be potential contributing factors. Among the most common synthetic OCs measured in marine mammal tissues are polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), polybrominated diphenyl ethers (PBDEs), and hexachlorobenzene (HCB). This project's focus was on the relationship between OCs and the western stock's lack of recovery. A suite of OCs were quantified from 239 hot-branded pups from 2001 – 2007 at nine Russian Far East rookeries. The use of brand-resighting data provided the opportunity to contrast pup survival, movement, reproductive success, and age at first reproduction between rookeries and among individuals with varying post-natal loads of OCs. Survival and movement were not affected by OC concentrations, but the estimated probability of survival within the first year was lower than expected at some rookeries. The effects of OCs on reproduction were less clear and no consistent pattern of negative effects emerged. Rookery specific differences indicated that location may be an important variable when considering survival, movement, and reproduction.

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General Introduction

Steller sea lions (*Eumetopias jubatus*) range from California's Channel Islands to northern Japan, with the western Steller sea lions (SSL) Distinct Population Segment occurring west of 144° longitude. The newly proposed Asian stock ranges west of 165° East longitude encompassing the majority of the Russian Far East SSL rookeries (Baker et al. 2005). The SSL population has declined by approximately 80% since 1976 and about 70% since 1985 (Myers et al. 2008; Sease et al. 2001; Calkins et al. 1999). The SSL was listed as endangered in 1997 under the Endangered Species Act (62 U.S. Federal Register 24345). The failure of the stock to recover to the levels set forth by the U.S. National Marine Fisheries Service Recovery Plan (NMFS 2008) has caused debate as to what may be preventing the recovery of the SSL. The apparent reduction in natality reported by Holmes et al. (2007) for the SSL was critical to the NMFS determination in the draft Biological Opinion (August 2010) that groundfish fisheries in Alaska may be jeopardizing the SSL recovery. Nonetheless, many researchers have commented on the importance of also looking at contaminants as contributing factors (Wang et al. 2011; Atkinson et al. 2008; Myers et al. 2008; NMFS 2008; Barron et al. 2003). The presence of persistent organohalogen contaminants (OCs) in the habitats of SSL may influence their vital rates, such as survival and reproduction (Noonburg et al. 2010; Huntington 2009; Tanabe 2002).

The effects of both anthropogenic and natural factors may have combined to prevent the successful recovery of the SSL. Anthropogenic-related contamination has not been ruled out as a significant cause of the failure of SSL to recover (Atkinson et al.

2008; Barron et al. 2003). Among the most abundant synthetic toxins measured in the tissues of SSL are OCs, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and dichlorodiphenyltrichloroethane (DDT), (Wang et al. 2011; Myers et al. 2008; Barron et al. 2003).

Contaminants and Toxicity

Determining the toxicity of such OCs in marine mammals is based on toxic equivalencies and their corresponding toxic equivalency factors as determined by the World Health Organization (Van den Berg et al. 2006, 1998). To establish the equivalency factor values, a relative effect potency is determined for individual chemicals by comparing their toxic and biological effects to the reference compound 2,3,7,8-tetrachlorodibenzo-p -dioxin, which was done primarily by laboratory research using mice and mink (Van den Berg et al. 2006, 1998). These values do not establish specific toxic thresholds for individual species, but do provide relative effect potencies and toxic equivalency factors for mammals, birds, and fish, which can help to establish thresholds for a given species. Thresholds will vary depending on what structure or function is being studied, such as reproductive failure, morbidity, carcinoma, and bone deformation. A suggested PCB threshold, expressed as lipid weight (lw) concentration, of 11,000 ng g^{-1}, and a wet weight (ww) concentration of 440 ng g^{-1}, measured in the liver or blood has been proposed for marine mammals (Kannan et al. 2000). There is, however, currently no biological threshold concentration identified for DDT contaminants in marine mammals (Kannan et al. 2004).

Organohalogen contaminants typically bioaccumulate in the food chain, are fat soluble (lipophilic), and resist metabolic and environmental breakdown (Myers et al. 2008; Becker 2000; Lee et al. 1996). The most common use of PCB has been for machinery lubricants and as coolants for electrical equipment, DDT was primarily used for agricultural activities, such as insecticides; however, their use has been banned in most developed countries since the 1970s and 1980s (Myers et al. 2008; Lee et al. 1996). These chemicals are insidious in the Arctic because of atmospheric and oceanic transport from Eurasia and tropical regions, which still utilize a suite of these chemicals (Aguilar et al. 2002; Bard 1999; Iwata et al. 1994, 1993). The transport of OCs to northern latitudes is promoted by atmospheric distillation and cold air sinks that permit these compounds to be deposited in the North Pacific habitats occupied by the SSL (Li et al. 2002; Bard 1999; Iwata et al. 1994, 1993).

The average OC concentrations measured in tissue samples were significantly greater in SSL from the Russian Bering Sea than those from western Alaska, specifically PCBs (Wang et al. 2011; Myers et al. 2008; Lee et al. 1996). In SSL from the western North Pacific, ~55% of the sample population (29 out of 52) had PCB congener concentrations greater than 14 ng g^{-1} lw of PCB 170 and 43 ng g^{-1} lw of PCB 180 (Hoshino et al., 2006); these concentrations of PCB congeners were suggested to cause a decrease in the circulating thyroid hormone (T3) in ribbon seals (*Phoca fasciata*) from the same region (Chiba et al. 2001).

Exposure to OCs has adverse effects on reproduction and endocrine functions in mammals (Van den Berg et al. 2006, 1998). Harbor seals (*Phoca vitulina*) fed a diet of herring from the Baltic Sea, which in that study was considered contaminated as compared to herring from the Atlantic Ocean, had decreased immune system response than those fed a diet of uncontaminated Atlantic Ocean herring (Ross et al. 1995). Wang et al. (2007a) showed that adult male harbor seals had higher mean levels of OCs than did females and that nursing dams may retain the more toxic non-*ortho* PCBs, thereby attenuating some of the toxic effects experienced by pups (Wang et al. 2007a,b). California sea lions (*Zalophus californianus*) may suffer from increased cancer-related mortality due to high concentrations of PCB contaminants (Ylitalo et al. 2005), and northern fur seal pups (*Callorhinus ursinus*) from primiparous dams had greater concentrations of OCs than pups from multiparous dams. This resulted in decreased immune system response, putting neonates more at risk of morbidity and mortality (Beckmen et al. 2003, 1999). Hawaiian monk seals (*Monachus schauinslandi*) accumulated OCs primarily in their blubber with adult males having the highest concentrations, and OCs in blood samples for 12 of 144 animals exceeded the recommended threshold for the sum of all quantified PCB contaminants, ∑PCB of 8.7 $\mu g\ g^{-1}$ lw (Ylitalo et al. 2008; Wilcox et al. 2004; Kannan et al. 2000). Most recently, DDT was measured in Galapagos sea lion (*Zalophus wollebaeki*) pups at concentrations known to cause anti-androgenic effects in other vertebrates (Alava et al. 2011). The inability to perform laboratory experiments on most marine mammals, as well as the complexity of contaminant mixtures, adds to the difficulty of ascertaining a discrete cause and effect relationship between contaminants and biological functions.

In Arctic species, chlorinated contaminants accumulate in fatty tissues (Becker 2000; Watanabe et al. 1999), undergo vertical transfer to offspring (Beckmen et al. 2003, 1999), and increase in concentration at higher trophic levels (Tanabe 2002; Beckmen et al. 1999; Watanabe et al. 1999). The total amount of contaminants in an organism is referred to as body burden. Both PCB and DDT levels, measured in archived marine mammal blubber, were at their highest concentrations during the 1970's, while DDT levels declined to one thirtieth of the greatest measured value by the 1990's and PCB levels declined to approximately half of their 1970 levels by the 1980's and 1990's (Tanabe et al. 2002). The years with the greatest levels of contaminants correspond with the overall reduction in SSL populations and subsequent US Endangered Species Act listing. Letcher et al. (2010) recommended a general threshold level of 1 ppm (1 ppm = 1000 ng g^{-1}) for any organohalogen contaminant or persistent organic pollutant in any tissue for marine mammals as an indication of high risk for negative biological effects.

Bering Sea SSL were identified as a species with considerable accumulations of OCs (Myers et al. 2008; Lee et al. 1996), for the sum of all quantified PCBs (∑PCB) and the sum of all quantified DDT contaminants (∑DDT). In Russia, ∑DDT in pups averaged approximately 8,364 ng g^{-1} lw, and for ∑PCB, 29% of Russian and 12% of Alaska pups, measured in whole blood from 2002 (Myers et al. 2008), exceeded the 11,000 ng g^{-1} lw threshold for ∑PCBs proposed for seals by Kannan et al. (2000), as well as the 1,000 ng

g^{-1} threshold proposed by Letcher et al. (2010). These concentrations are lower than those reported by Lee et al. (1996) for SSL from Alaska and the Russian Bering Sea, sampled from the late 1970's – 1981, than those reported by Myers et al. (2008). However, different analytical techniques were used to quantify OCs, so a direct comparison would be inappropriate, but does show that SSL in these regions have considerable accumulations of OCs. The range of ∑PCB was from 5,700 to 41,000 ng g^{-1} lw for males and from 570 to 16,000 ng g^{-1} lw for females; ∑DDT values ranged from 2,800 to 17,000 ng g^{-1} for males and from 190 to 6,500 ng g^{-1} lw for females measured in the blubber and liver (Lee et al. 2006).

Male SSL likely experience an increasing body burden of contaminants with age, whereas females tend to show increasing levels followed by a sharp decrease after parturition. It is estimated that approximately 80% of PCBs and 79% of DDTs of female body burden are transferred through lactation (Lee et al. 1996). This suggests that pups of primiparous dams will be exposed to high levels of contaminants, as was demonstrated in northern fur seals (Beckmen et al. 2003, 1999).

Research Objectives

The goal of the present study was to examine the potential relationship between PCBs, DDTs, and PBDEs and the survival, movement, and reproduction of SSL. To address these research objectives the following three hypotheses were tested: H_1: There is no difference in pup survival and movement probabilities among rookeries or between pups above and below the mean post-natal OC concentrations, H_2: Age at first reproduction is not associated with post-natal contaminant concentrations, and H_3: Breeding success is not associated with post-natal contaminant concentrations. The OCs quantified were 15 PCB congeners (77, 101, 126, 105, 118, 169, 128, 189, 153, 138, 157, 170/194, 156, 180), 6 DDT metabolites (o,p'-DDD, p,p'-DDD, o,p'-DDE , p,p'-DDE, o,p'-DDT, p,p'-DDT), HCB (hexachlorobenzene), and 8 PBDE congeners (3, 15, 28, 47, 99, 154, 153, 183). Whole blood and serum samples analyzed for OCs were taken from 239 hot-branded SSL pups at nine Russian Far East rookeries (Iony Island, Yamsky, Tuleny, Brat Chirpoyev, Srednego, Lovushki, Antsiferov, Kozlova Cape, and Medny Island) from 2001 - 2007. The intensive brand-resight effort conducted on Russian rookeries provided the opportunity to contrast pup survival, movement, reproductive success, and age at first reproduction among individuals with varying post-natal concentrations of OCs. A series of mark-recapture multi-strata models in program MARK were used to estimate survival, resighting, and movement probabilities as a function of contaminant concentration, location, and age. Linear models were used to test for correlations between female reproductive success and age at first reproduction to OCs and natal rookery.

Chapter 1: The effect of environmental contaminants on western Steller sea lion survival and movement estimated from multi-state mark-recapture methods

Abstract

The western Distinct Population Segment of Steller sea lions (*Eumetopias jubatus*) has experienced dramatic declines since the 1960's; particularly in the western Alaskan and Asian portions, which have continued to decline or stabilized at low levels. Causes for this decline have not been identified, but may include anthropogenic contamination from organohalogen contaminants (OCs). These include polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT), which have not been ruled out as a potential cause for the lack of recovery. The objective of this study was to determine the effects of OCs on survival and movement probabilities estimated in program MARK using resighting data collected from 2003 -2009. PCBs and DDTs were measured from 136 (74 males and 62 females) individually marked, free-range pups from four Russian Far East rookeries. Survival and movement were most affected by age and location rather than OCs. The lowest estimated probabilities of survival occurred in the first year, ranging from 38% - 74%, but increased for individuals between ages 1 and 9, ranging from 82% - 94%. The greatest emigration occurred from Medny Island west toward the Kamchatka Peninsula (33%) and to Bering Island (18%). The estimated probabilities of resighting

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varied by location (48% - 87%), but had greater precision than survival or movement parameters. Survival probabilities were lower than expected within the first year, which may indicate rookery specific dynamics and point to locations at risk of continued declines.

Introduction

Steller sea lions (*Eumetopias jubatus*) range from California's Channel Islands to northern Japan, with the western Steller sea lions (SSL) Distinct Population Segment occurring west of 144° longitude. The newly proposed Asian stock ranges west of 165° east longitude encompassing the majority of Russian Far East SSL rookeries (Baker et al. 2005). The SSL population declined by approximately 80% since 1976 and about 70% since 1985 (Myers et al. 2008; Sease et al. 2001; Calkins et al. 1999). The SSL was listed as endangered in 1997 under the Endangered Species Act (62 U.S. Federal Register 24345). The apparent reduction in natality reported by Holmes et al. (2007) for the SSL was critical to the NMFS determination in the draft Biological Opinion (August 2010) that groundfish fisheries in Alaska may be jeopardizing SSL recovery. The failure of the stock to recover to the levels set forth by the U.S. National Marine Fisheries Service Recovery Plan (NMFS 2008) has caused debate as to what may be preventing the recovery of SSL, and many researchers have commented on the importance of researching contaminants as causative factors (Wang et al. 2011; Atkinson et al. 2008; Myers et al. 2008; NMFS 2008; Barron et al. 2003). The presence of persistent organohalogen contaminants (OCs) in habitats of the SSL could possibly influence their survival (Noonburg et al. 2010; Huntington 2009; Tanabe 2002).

Anthropogenic-related contamination is being investigated as a potentially significant cause of the failure of SSL to recover (Atkinson et al. 2008; Barron et al. 2003). Among the most abundant synthetic toxins measured in the tissues of SSL are OCs, such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDTs) (Wang et al. 2011; Myers et al. 2008; Barron et al. 2003), and their concentrations can vary in an individual over time (Myers and Atkinson 2012). Common uses for PCBs include use as machinery lubricants and as coolants for electrical equipment, and DDTs were commonly used as agricultural insecticides; however, their use has been banned in most developed countries since the 1970s and 1980s (Lee et al. 1996). These chemicals are insidious in the Arctic because of atmospheric and oceanic transport from Eurasia and tropical regions which still utilize a suite of these chemicals (Aguilar et al. 2002; Bard 1999; Iwata et al. 1994, 1993). The transport of OCs to northern latitudes is promoted by atmospheric distillation and cold air sinks that permit these compounds to be deposited in the North Pacific habitats occupied by the SSL (Li et al. 2002; Bard 1999; Iwata et al. 1994, 1993).

When fat soluble (lipophilic) OCs enter the environment they typically bioaccumulate in the food chain and resist metabolic and environmental breakdown (Myers and Atkinson 2012; Myers et al. 2008; Becker 2000; Lee et al. 1996). Both PCB and DDT concentrations measured in archived northern fur seal (*Callorhinus ursinus*) fat tissues were highest during the 1970's (Tanabe et al. 1994). However, concentrations of DDTs declined to one thirtieth of their greatest measured value by the 1990's and concentrations of PCBs declined to approximately half of their 1970 levels by the 1980's and 1990's (Tanabe 2002). The years with the greatest concentrations of contaminants correspond with the years of overall decline in SSL populations and subsequent U.S. Endangered Species Act listing. A threshold level of 1ppm (1ppm = 1000 ng g^{-1}) for any organohalogen contaminant or persistent organic pollutant in any tissue for marine mammals as an indication of high risk for negative biological effects was recommended by Letcher et al. (2010). Similarly, a PCB threshold, expressed as lipid weight (lw) concentration of 11,000 ng g^{-1}, and a wet weight (ww) concentration of 440 ng g^{-1}, measured in the liver or blood was proposed by Kannan et al. (2000). These values were derived from dead and stranded marine mammals, not including SSL. There is, however, currently no biological threshold concentration identified for DDT contaminants in marine mammals (Kannan et al. 2004).

Exposure to OCs was shown to have adverse effects on reproduction and endocrine functions in mammals (Van den Berg et al. 2006, 1998). For example, in SSL from the western North Pacific, ~55% of the sample population (29 out of 52) had PCB concentrations greater than 14 ng g^{-1} lw of PCB 170 and 43 ng g^{-1} lw of PCB 180 (Hoshino et al. 2006); these concentrations of PCB congeners were suggested to cause a decrease in the circulating thyroid hormone (T3) in ribbon seals (*Phoca fasciata*) from the same region (Chiba et al. 2001). California sea lions (*Zalophus californianus*) may suffer from increased cancer-related mortality due to high concentrations of PCB contaminants (Ylitalo et al. 2005). Blubber and liver samples collected from Alaskan SSL from 1976 – 1978 had PCB concentrations that were one to two orders of magnitude higher than in other Arctic pinnipeds, such as, harp seals (*Pagophilus groenlandicus)*, ringed seals (*Phoca hispida*), and northern fur seals, and similar results, but overall lower concentrations were measured for DDT (Lee et al. 1996).

The objective of the present study was to use OC measurements from 136 individually marked Steller sea lion pups to determine probabilities of resighting, movement, and survival at four Russian Far East rookeries. A series of mark-recapture multi-strata models in program MARK were used to estimate these probabilities. Resighting probability was estimated for each location using days of effort as a covariate. The null hypotheses tested were: there is no difference in pup survival and movement probability among rookeries or between pups above and below the mean OC concentrations. To test these hypotheses, whole blood samples were collected in 2002 from free-ranging SSL pups in the Russian Far East and analyzed for DDT and its metabolites and a suite of PCB congeners. Contaminant concentrations were measured on a whole blood wet weight basis, which Myers and Atkinson (2012) have suggested is appropriate for quantifying OC levels in SSL. From the resighting data, we estimated survival, movement, and resighting probabilities.

Methods

Sample Collection

Field efforts were conducted to individually mark SSL pups, collect blood samples for health assessments, and survey rookeries in the Russian Far East (Figure 1.1). The study area encompassed a segment of the endangered western and newly proposed Asian SSL stocks. In late June to early July 2002, whole blood samples were collected for contaminant analysis from 136 hot-branded free-ranging SSL pups approximately one month old (Myers et al. 2008). Samples were collected from four rookeries: Iony Island (females n=12, males n=14), Kozlova Cape (females n=17, males n=21), Medny Island (females n=21, males n=18), and Yamsky (females n=13, males n=20).

The procedure for individually marking SSL pups followed the methods of Merrick et al. (1996) for applying unique alphanumeric hot-brands on SSL, which remain legible for at least 7 years (Merrick et al. 1996). Prior to hot-branding, individuals were anesthetized using mobile isoflurane following the protocols of Hastings et al. (2009) and Heath et al. (1996). All samples and corresponding data were collected during the 2002 field season and were maintained by North Pacific Wildlife Consulting in collaboration with the National Marine Mammal Laboratory (Myers et al. 2008).

Blood samples were drawn from a rear flipper or caudal-gluteal vein and stored at -80 ^{o}C for later chemical analyses as described in Myers et al. (2008). Quantification of 15 PCBs (77, 101, 105, 118, 126, 128, 138, 153, 156, 157, 169, 170/194, 180, 189) and six chlorinated contaminants (o,p’-DDD, p,p’-DDD, p,p’-DDE, o,p’-DDT, p,p’-DDT, hexachlorobenzene), were measured using high-performance liquid chromatography/photodiode array; these concentrations were previously published in Myers et al. (2008). The total concentration of PCBs was calculated by summing all 15 PCB congener concentrations (∑PCB), and similarly for ∑DDT. Total lipid quantities in each whole blood sample were measured by thin layer chromatography with flame ionization detection (Myers et al. 2008). The present study is an extension of the Myers et al. (2008) research. This project uses the OC measurements from Myers et al. (2008) and incorporates the resighting data from those SSL pups to determine survival, movement, and resighting probabilities.

Resighting histories of 136 hot-branded SSL pups born in 2002 were obtained by photo-documentation during June – December from 2003 – 2011. Resighting effort was conducted at major rookeries and haulout sites across the Russian Far East during boat-based surveys (Table 1.1). Branded SSL were photographed from small boats, land-based vantage points, and field camps at Kozlova Cape, Medny Island, Tuleny, and Antsiferov rookeries. Kozlova Cape and Medny Island used remote video and still camera systems maintained by personnel at nearby field camps. The remote monitoring systems at these locations were described in a previously published study (Burdin et al. 2009). Only those resightings accompanied by a confirmed image of the brand were used as confirmed animal sightings by North Pacific Wildlife Consulting.

Model Estimates

Probability estimates for survival, resighting, and movement were calculated in program MARK, version 6.1, using a multi-state live recaptures model. This project used resighting histories from 136 hot-branded SSL pups from four natal rookeries (Iony Island, Kozlova Cape, Medny Island, and Yamsky) that were identified at up to 13 different locations within the study region from 2003 – 2011. Within a single survey year some individuals were seen at up to four different locations. Therefore, resighting locations were grouped into four different regions, or strata, that included up to two of the four natal rookeries (Figure 1.2). We used four different strata in our analyses by using the locations at which SSL were resighted based on their natal rookeries. Iony Island and Yamsky were included in stratum A, which also included Tuleny and Antsiferov rookeries, being the two other locations SSL born on Iony Island or Yamsky were resighted; Kozlova Cape is stratum B, which includes five haulout locations along the west coast of the Kamchatka Peninsula at which SSL born on Kozlova Cape were resighted; Bering Island is stratum C, which includes a total of four haulout locations without a rookery; and Medny Island is stratum D, which includes a rookery and two haulout locations. Resighting effort, measured in days, was summed across all locations within each stratum from 2003 – 2011 and used as a covariate for recapture probability. Capture histories for 10 occasions (2002 – 2011), for all SSL, were created using binary coding (0 = not resighted, 1 = resighted). Multiple observations of an individual within a year were considered as one observation based on time observed at each location, where the location that the individual was observed the most was considered the resighting location. This was done for computational ease in the model analyses.

"Apparent survival", (ϕ), was estimated for each stratum, as well as for those animals above and below the group mean OC level within and between each stratum. In program MARK apparent survival is estimated as survival multiplied by the probability of that individual remaining in the study area, such that if animals move outside of the study area, a biased survival estimate results; therefore we calculated "apparent survival" and not true survival (Cooch and White, 2011). Contaminants (∑PCB & ∑DDT) were used as covariates for estimating survival probability in alternate models. The probability of recapture (p) for a marked SSL was determined by that individual surviving, ϕ, from time $t \rightarrow t + 1$ and being encountered. The probability of movement (Ψ) was dependent on the marked individual surviving, transitioning between strata, and being encountered (Cooch and White, 2011). The formula considering all three of these parameters for a marked SSL from stratum a at time t transitioning to, and being encountered in, stratum b at time $t + 1$ is $\phi_{b,t+1} = \phi_{a,t}\ \Psi_{a\text{-}b,t}\ p_{b,t+1}$. The assumptions in multi-state models are that survival from time t to t +1 does not depend on the strata at time t +1, that individuals make transitions at the same time, and that the distribution of transitions is known (Cooch and White, 2011). For the purposes of this study we accepted the first assumption of non-dependent survival at time t +1 and that animals were making transitions at similar times within the summer breeding season. By using locations from resighting histories, we know the distribution of their transitions, which were included in the strata framework of the models.

A stepwise modeling approach was used to determine interactions between contaminants survival and movement. We allowed survival (ϕ) to be constant across strata or to vary by age, strata, and mean OC concentration. We also varied the movement parameter (Ψ) by two age groups (0, 1+), age 0 is the initial capture and hot-branding year in 2002 and age 1+, wherein resighting ranged from 2003 – 2011. The age variable was separated into the same two groups for estimating survival and movement probabilities. Contaminants were included in the models as groups, determined by pups having OC concentrations greater or less than the overall mean OC concentrations. This approach gave us eight reasonable models to compare and to test the hypothesis that there was no difference in pup survival among rookeries or between pups above and below the mean OC concentrations. Grouping SSL above and below the mean OC concentrations was determined by separately calculating the mean for ∑PCB and ∑DDT and using that mean to define SSL as being above or below that value. There was one SSL that was below the group mean for ∑PCB but above for ∑DDT and five SSL that were below the group mean for ∑DDT but above for ∑PCB. Because these SSL were above the mean concentration for at least one of the contaminants they were classified as being above the group mean OC concentrations.

An alternative set of models was used to test for differences between either ∑PCB or ∑DDT on survival because of a strong correlation between ∑PCB and ∑DDT (r = 0.88). Each model tested included recapture probability with resighting effort in days as a covariate for each stratum. However, stratum C in our design was not a natal rookery where pups were branded, so ϕ was not estimated for this region. Alternatively, stratum C survival estimates were set constant ($\phi = 0.66$ for age 0 and 0.86 for age group 1+), which was a reasonable average probability for SSL in that region at age 0 and 1- 9, respectively (Burkanov, unpublished results). However, both movement and resighting probability can be estimated for stratum C based on resighting history over the last nine years.

Model comparison and selection were determined using AICc (Akaike Information Criterion, second order) and the difference (Δ) between AICc for a given model and the model with the lowest AICc. Model support is based on the Kullback-Leibler distance between models represented as ΔAICc, where the larger the Δ the less support there is that the model adequately explains variation in the data (Burnham and Anderson, 2002). A Δ range from 0 – 2 provides strong support for the model being the best model, Δ 2 – 4 indicates weak support for the model not being best model, Δ 4 – 7 indicates moderate support for the model not being the best model. If the model considered has a Δ 7 – 10 then it provides strong evidence that the model is not the best model and a $\Delta > 10$ very strongly indicates that the model is not the best model and fails to adequately explain a considerable amount of variability (Burnham and Anderson, 2002).

For the primary analysis, the survival parameters were separated into two age groups; however, a suite of models were also tested using multiple age-varying scenarios. The models that tested alternate age scenarios resulted in ΔAICc > 1000 and a deviance two to six times greater than models where age was grouped into age 0 and age 1+.

Models were of the form, ϕ (a,s,c), p (s,eff), and Ψ (a,c), for which *a* is age, *s* is stratum, and *c* is mean OC concentration. There were 14 biologically meaningful models tested; 10 that used OC concentrations to group SSL above or below the group mean for ∑PCB and ∑DDT (Table 1.2), and four that analyzed OCs as individual covariates on survival (Table 1.3).

Results

The average whole blood concentration for ∑PCB was 4.25 ± 5.12 ng g^{-1} ww (n = 136). For SSL grouped above the aggregate mean the average concentration was 9.25 ± 6.55 ng g^{-1} ww (n = 44), and below the aggregate mean it was 1.86 ± 0.89 ng g^{-1} ww (n = 92) (Figure 1.3). The average whole blood concentration for ∑DDT was 3.22 ± 4.28 ng g^{-1} ww. For SSL grouped above the aggregate mean the average concentration was 7.65 ± 5.21 ng g^{-1} ww, and below the aggregate mean it was 1.11 ± 0.65 ng g^{-1} ww (Figure 1.3). For the one SSL that was above for ∑DDT and not ∑PCB, the concentrations were 3.6 ng g^{-1} ww and 3.1 ng g^{-1} ww, respectively. For the five SSL that were above for ∑PCB and not ∑DDT, the average concentrations were 4.62 ± 0.19 ng g^{-1} ww and 2.80 ± 0.29 ng g^{-1} ww, respectively.

Model comparison statistics, such as AICc, are shown in Table 1.2 for the models considered to estimate survival, resighting, and movement probabilities. These include models where SSL were grouped by being either above or below the overall average OC concentration and age was grouped by age 0 and age 1+ SSL. Model 4 results are presented to illustrate that OCs have an equivocal affect on both the survival and movement parameters.

The probability of survival was also estimated where ∑PCB and ∑DDT were used as covariates (Table 1.3). Models 15 and 16 show two alternate age-varying models, such that age in model 15 was separated into two groups, from birth to two years of age and from two to nine years of age and age in model 16 the two groups were from birth to three years of age and from three to nine years of age. The ΔAICc (> 10) for these models indicates that less model variability is explained when compared to models 11- 14. The ΔAICc (< 2) between model 11, which includes all OCs as covariates, and model 13, which has no OC covariates, indicates that including OCs does not explain any more variability in the data.

Estimations for the probability of survival from model 4 did not clearly indicate that OC concentrations above (c_g) or below (c_l) the average concentration affect SSL differently (Table 1.4). Overall, survival was most different between strata and age groups. The greatest difference within a stratum and age group was for age 0 SSL in stratum B (Kozlova Cape) with estimated survivals ϕ (0, B, c_g) = 0.74, with a 95% confidence interval (0.56, 0.86) and standard error of 0.08, and ϕ (0, B, c_l) = 0.56, with a 95% confidence interval (0.49, 0.63) and standard error of 0.04. Kozlova Cape is also the site of the highest overall OC concentrations of the four strata (Myers et al. 2008). Stratum A had the lowest survival estimates for age 0, ϕ (0, A, c_g) = 0.38 and ϕ (0, A, c_l) = 0.41. However, survivals at age 1+ for stratum A, ϕ (1+, A, c_g) = 0.89 and ϕ (0, A, c_l) =

0.87, were nearly equal to those for age 1+ for stratum D, ϕ (1+, D, c_g) = 0.90 and ϕ (1+, A, c_l) = 0.83. The lowest survival probabilities in each stratum occurred at age 0, ranging from 0.38 ± 0.07 in stratum A to 0.74 ± 0.08 in stratum B.

The probabilities of resighting were estimated with greater precision than survival probabilities indicated by reduced standard errors, 1% - 2%, and narrower confidence intervals (Figure 1.4). The greatest probability of resighting was for stratum C (p = 0.87) followed by stratum B (p = 0.75), D (p = 0.63), and A (p = 0.48). The wide confidence interval for stratum A (0.44, 0.52) is likely due to multiple years of no resighting effort on Yamsky and Iony Island rookeries.

The greatest probabilities of movement occurred between strata B, C, and D in the eastern portion of the study region (Figures 1.5, 1.6). Movement from stratum A to other strata was minimal for any age group, with a maximum Ψ = 0.02 for Ψ (1+, A $\rightarrow$ C, c_l), data not shown. The probabilities of movement for age 0 SSL (Figure 1.5) were from their natal rookery to all locations in Figure 1.2. Steller sea lions from stratum D had a greater probability of moving to stratum B (Ψ = c_g 0.33, c_l 0.13) than to any other location and SSL from stratum B were seen less in other strata (Ψ = c_g 0.04, c_l 0.11) (Figure 1.5).

For age 1+ SSL there was less movement between strata B and D, but greater movement to and from stratum C (Figure 1.6) when compared to age 0 SSL. The probabilities of movement for age 1+ SSL were from their natal rookery to all locations shown in Figure 1.2. The most frequent movements occurred from stratum C to D (Ψ = c_g

0.40, c_l 0.31) and to stratum B (Ψ = c_g 0.19, c_l 0.40) (Figure 1.5). Movement between strata B and D for age 1+ SSL (Ψ range = 0.01 - 0.07) was less compared to the age 0 SSL, but had increased probabilities of movement from stratum C to A (Ψ range = 0.04 - 0.40) (Figure 1.5). Contaminant concentrations failed to explain any differences in movement probabilities between groups above or below average OC concentrations (Table 1.5).

Discussion

The results for model ϕ (a,s,c), p (s,eff), Ψ (a,c) were presented to show the absence of a detected effect of contaminants on survival (ϕ) and movement (Ψ) in terms of those individuals above and below mean contaminant concentrations. The ΔAICc between model 4 and model 1, which had the best fit was 10.74 (Table 1.2), which strongly suggests that model 4 is not likely to be the best model (Burnham and Anderson, 2002). Parameter estimates compared between these models, but excluding contaminants, resulted in survival and resighting probability estimates within 5% of each other, except for survival at stratum B (0, B, c_g), which was ~15% lower in the better fit model 1. This estimate had a high standard error and wide confidence interval, suggesting no contaminant effect. The estimates of movement probability changed more dramatically when the OC group was excluded, as in model 1 (Table 1.2), resulting in a reduced AICc with six fewer parameters estimated for survival (ϕ) and 14 fewer parameters estimated for movement (Ψ). For all models the overdispersion parameter, $\hat{c} > 1$, indicated overdispersed data. The cause of overdispersed data comes from the estimated variance being greater than expected from a binomial model with independence among individuals (Burnham and Anderson, 2002). This result can be expected for a biological model with a sample size of n = 136, with each individual having a highly variable encounter history.

All model estimates indicated that SSL survival is influenced more by age and location than by contaminant load. The greatest risk of mortality for all rookeries occurred within the first year but decreased by age nine. The lowest survival probabilities were seen in stratum A (Iony Island, Yamsky, Tuleny, Antsiferov) for both age groups with the exception of age 1+ SSL when compared to the lower estimate in stratum D (Medny Island) of age 1+ SSL below mean OC level. However, the estimates for stratum A were likely underestimated due to poor resighting effort on Iony Island and Yamsky rookeries. Both Kozlova Cape and Medny Island rookeries had the greatest estimated survival for age 0 SSL and their populations appeared to have stabilized at depressed levels with some signs of increase (Burkanov et al. 2011, 2005).

Results did not consistently show that those pups with more contaminants had a lower probability of survival. The stratum B age 0 group with lower contaminant concentrations had a much lower survival probability at age 0 when compared to the more contaminated group (Table 1.4); however, the less contaminated group had half the standard error and a confidence interval nearly half the range of that of the more contaminated group. This suggests that apparent differences between the ϕ (0, B, c_g) and ϕ (0, B, c_l) estimates could be an artifact due to variability in parameter estimation. There was a similar relationship for the stratum D age 1+ group. The more contaminated SSL had a probability of survival ~7% higher than those with less contamination (Table 1.4). This result suggests that SSL with more contaminants had a greater probability of survival at age 1+, which also occurred in stratum B for age 0 SSL. Since it is unlikely that OCs had a positive effect on survival, there is likely a missing explanatory variable needed to understand the difference between the two contaminant groups at strata B and D. Additional data that could broaden our understanding of this contradiction could come from longitudinal blood sampling from the same individual or from adults to better establish baseline OC concentrations and fluctuations over time.

The SSL pups in our study each had OC concentrations lower than the suggested contamination threshold, likely resulting in no effect of OCs on the probability of survival or movement. The greatest concentration measured in our study was 36 ng g^{-1} ww measured at Kozlova Cape in a male pup resighted the year after branding in 2003 and from 2007 – 2011. This OC value is much lower than the reported threshold value of 440 ng g^{-1} ww (Kannan et al. 2002), which is expected to have negative biological effects, such as decreased immune system function (Beckmen et al. 2003; Ross et al. 1995). However, this is a single measurement taken during a vulnerable stage in pup development, and in a pup that was still suckling and continued to be exposed to its mother's contaminants until weaned. Pups that are receiving nutrients only from suckling may receive up to 79% of PCBs and 80% DDTs of the female's total body burden of contaminants (Lee et al. 1996) and some SSL pups may continue to suckle periodically for up to 4 years (Mamaev and Burkanov, 2004).

Estimates for the probability of resighting were calculated with greater precision than those for survival and movement, evidenced by narrower confidence intervals and smaller standard errors (Figure 1.3). Steller sea lions from stratum D (Medny Island) frequently used stratum C as a haulout location as well as SSL from stratum B (Kozlova Cape) but less often. This resulted in stratum C having the highest resight probability (p = 0.87) despite not being a natal rookery. Both strata B and D used remote monitoring systems, which increased the probability of sighting a SSL. The populations at strata B and D appearred to be stabilized at a low level (Burkanov et al. 2005), where pup production can be highly variable between years, but dams do show strong site fidelity (Burkanov, personal communication). This suggests that if an individual was alive, it was likely to be resighted during its lifetime.

Resighting probability for stratum A (Yamsky, Iony Island, Tuleny, and Antsiferov) had wider confidence intervals than other strata due to poor resighting effort on Yamsky and Iony Island (Figure 1.3). Yamsky and Iony Island are at the extreme northern end of observed rookeries in the Sea of Okhotsk, such that lack of funding and personnel has limited access to those areas. This has resulted in large variability in encounter histories for SSL born on Yamsky and Iony Island rookeries due to low sample sizes. Because of the inclusion of Tuleny and Antsiferov in stratum A the estimates for probability of resighting were likely biased higher for pups branded on Yamsky and Iony Island then were they to be estimated separately from Tuleny and Antsiferov.

To estimate the probability of movement and to avoid inestimable results between strata where there was little to no movement, a range of parameter values were set to zero. The movement parameters set to zero and not estimated were from stratum A to B, and for age 0 from stratum A to C and D, from stratum B to C and D, from stratum C to A, B, and D, and from stratum D to A. These specific Ψ parameters had to be set to zero because of standard errors that included 0 or 1, unreasonably small probability estimates (10^{-24}), and a ΔAIC > 10 when compared to the same model in which the Ψ parameters were set constant to zero. Age 0 animals did not have limited movement in all cases; there was an estimated 33% probability of age 0 SSL born on stratum D (Medny Island) moving to stratum B (Kozlova Cape) within their first year. The greatest estimated probabilities of movement occurred along the east coast of Kamchatka from stratum C (Bering Island) to B and D for age 1+ SSL (Figure 1.4). The lowest estimated probabilities were for stratum A (Iony Island and Yamsky), which was at the northern most extent of SSL rookeries in Russia (Burkanov et al. 2011). Stratum B was the most traveled to location by SSL from all other strata presumably because it included a rookery and five haulout locations along the Kamchatka Peninsula. In contrast, stratum D was the location, which includes a rookery, which had the most emigration. This location is part of the Commander Islands and considered part of the endangered and declining western stock.

Model results indicate that including OCs as a variable for estimating Ψ (movement probabilities) did not reduce AICc and thus has little to no effect on an individual's ability to transition to other locations. If OCs were at or above threshold concentrations, then a reduced level of fitness may have been detected, possibly affecting an individual's ability to migrate.

The model estimates presented here must be interpreted with caution for several reasons. First, resighting effort varied by location, year, and method. However, we have attempted to account for some of this variability by using resighting effort as a covariate in our models. Additionally, some biasing certainly occurred between regions using different techniques for obtaining resighting data. Second, sample size was small (n = 136), so that many parameter estimates were imprecise. Third, contaminant concentrations represented only a single whole blood measurement that is being used to investigate relationships with survival, resighting, and movement probabilities over nine years. Therefore, our results only represented an initial step to uncovering the relationship of organohalogen contaminants on SSL survival, resight, and movement probabilities. Additional research should focus on obtaining multiple blood samples from individuals throughout their lifetime to measure changes in contaminant concentrations in conjunction with long term brand monitoring.

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Tables

Table 1.1 Resight effort, in days, by year, natal rookery, and haulout sites. The total column reflects the sum of effort over nine years beginning the year after initial branding in 2002.

	2003	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**Total**
Natal Rookeries										
Iony Is.	0	0	0	2	0	0	10	0	2	**14**
Kozlova Cape	56	53	101	78	63	3	26	77	79	**536**
Medny Is.	85	72	90	80	87	76	79	128	79	**776**
Yamsky	0	0	33	48	53	1	0	0	2	**137**
Haulout Sites										
Medny Is. Group	1	5	0	0	0	1	4	1	1	**13**
Tuleny	44	38	41	41	37	0	52	45	73	**371**
Avacha Bay	0	0	0	5	0	4	40	21	0	**70**
Kamesity Cape	0	0	0	0	0	1	0	0	0	**1**
Kekurny Cape	3	0	8	8	0	1	1	0	0	**21**
Shipunsky Cape	0	0	0	0	0	1	0	0	0	**1**
Zheleznaya Cape	0	0	2	0	0	1	0	0	0	**3**
Antsiferov	54	57	50	61	47	45	40	56	48	**458**
Bering Is. Group	10	74	44	45	40	18	72	53	43	**399**

Table 1.2. Fits of models in which contaminants are grouped above or below the average OC concentration. Models are identified based on the probability of survival (ϕ), capture (p), and movement (Ψ). For ϕ parameters: a = age (0, 1+), s = strata (A, B, C, D), and c = combined contaminants (PCB, DDT). Estimates of survival for stratum *C* are fixed, ϕ [C_0 = 0.66, C_{1+} = 0.86]. For p parameters: eff = resighting effort at each strata. K is the number of parameters; AICc, Akaike Information Criterion measuring model fit; ΔAICc, difference between AICc and the lowest model AICc; Deviance, measure of model fit; -ln L, log likelihood.

	Model	K	AICc	ΔAICc	Deviance	-ln L
1.	ϕ(a,s), p(s,eff), Ψ(a)	22	5320.52	0.0	2712.84	5275.98
2.	ϕ(a,s,c), p(s,eff), Ψ(a)	28	5324.32	3.80	2704.31	5267.45
3.	ϕ(a,s), p(s,eff), Ψ(a,c)	36	5327.32	6.80	2690.74	5253.88
4.	ϕ(a,s,c), p(s,eff), Ψ(a,c)	42	5331.26	10.74	2682.17	5245.30
5.	ϕ(a,c), p(s,eff), Ψ(a)	20	5368.37	47.85	2764.78	5327.92
6.	ϕ(a), p(s,eff), Ψ(a)	19	5372.64	52.12	2771.10	5334.23
7.	ϕ(a), p(s,eff), Ψ(a,c)	32	5373.13	52.60	2774.85	5307.99
8.	ϕ(a,c), p(s,eff), Ψ(a,c)	34	5375.28	54.76	2742.86	5306.00
9.	ϕ(.), p(s,eff), Ψ(a)	17	5580.18	259.65	2982.71	5545.85
10.	ϕ(.), p(s,eff), Ψ(a,c)	31	5585.98	265.46	2959.77	5522.91

Table 1.3. Fits of models in which contaminants are covariates. Models are identified based on the probability of survival (ϕ), capture (p), and movement (Ψ). For ϕ parameters: a = age (0, 1+) and PCB and DDT contaminants both individually and jointly. For p parameters: eff = resighting effort at each strata and s = strata (A, B, C, D). K is the number of parameters; AICc, Akaike Information Criterion measuring model fit; ΔAICc, difference between AICc and the lowest model AICc; Deviance, measure of model fit; -ln L, log likelihood.

	Model	K	AICc	ΔAICc	Deviance	-ln L
11.	ϕ(a,PCB,DDT), p(s,eff),Ψ(a)	22	1214.25	0.0	1167.74	1167.74
12.	ϕ(a,DDT), p(s,eff), Ψ(a)	20	1215.17	.92	1173.10	1173.10
13.	ϕ(a), p(s,eff), Ψ(a)	18	1215.44	1.19	1177.76	1177.76
14.	ϕ(a,PCB), p(s,eff), Ψ(a)	20	1217.34	3.08	1175.26	1175.26
15.	ϕ(a), p(s,eff), Ψ(a)	17	2572.42	1358.16	2536.92	2536.92
16.	ϕ(a), p(s,eff), Ψ(a)	14	6604.96	5390.71	6575.94	6575.94

Table 1.4. Estimates for the probability of survival including 95% confidence intervals (in parentheses) and standard error using Model 4: ϕ(a,s ,c), p(s,eff), Ψ(a,c). Stratum C ϕ estimates are fixed at [C_0 = 0.66, C_{1+} = 0.86] so are omitted below. Notation is such that $A_{0,}\ c_g$ = stratum A, age 0, above mean OC concentration and $A_{1+,}\ c_l$ = stratum A, age 1+, below mean OC concentration.

Strata	Probability of survival (ϕ)	Standard Error
$\phi A_{0,}\ c_g$	0.38 (0.25, 0.52)	0.07
$\phi A_{1+,}\ c_g$	0.89 (0.81, 0.94)	0.03
$\phi A_{0,}\ c_l$	0.41 (0.34, 0.48)	0.03
$\phi A_{1+,}\ c_l$	0.87 (0.84, 0.90)	0.02
$\phi B_{0,}\ c_g$	0.74 (0.56, 0.86)	0.08
$\phi B_{1+,}\ c_g$	0.91 (0.85, 0.94)	0.02
$\phi B_{0,}\ c_l$	0.56 (0.49, 0.63)	0.04
$\phi B_{1+,}\ c_l$	0.94 (0.92, 0.96)	0.01
$\phi D_{0,}\ c_g$	0.72 (0.55, 0.85)	0.08
$\phi D_{1+,}\ c_g$	0.90 (0.81, 0.95)	0.04
$\phi D_{0,}\ c_l$	0.73 (0.62, 0.82)	0.05
$\phi D_{1+,}\ c_l$	0.83 (0.76, 0.88)	0.03

Table 1.5. Estimates for the probability of movement including 95% confidence intervals (in parentheses) and standard error, SE, using model ϕ(a,s ,c), p(s,eff), Ψ(a,c). Probabilities are shown for Age 0 and Age 1+ above the mean (c_g) and below the mean (c_l) OC concentration.

Strata → Strata	Age 0, c_g	SE	Age 0, c_l	SE
B → A	0.04 (0.01, 0.24)	0.04	0.11 (0.06, 0.20)	0.03
D → B	0.33 (0.18, 0.53)	0.09	0.13 (0.07, 0.24)	0.04
D → C	0.05 (0.01, 0.27)	0.05	0.02 (0.00, 0.12)	0.02

Strata → Strata	Age 1+, c_g	SE	Age 1+, c_l	SE
A → C	0.02 (0.00, 0.07)	0.01	0.02 (0.01, 0.04)	0.01
A → D	0.02 (0.00, 0.07)	0.01	0.00 (0.00, 0.02)	0.00
B → A	0.01 (0.00, 0.05)	0.01	0.02 (0.01, 0.04)	0.01
B → D	0.03 (0.01, 0.08)	0.01	0.03 (0.02, 0.05)	0.01
C → A	0.14 (0.03, 0.41)	0.09	0.04 (0.01, 0.14)	0.03
C → B	0.19 (0.06, 0.46)	0.10	0.40 (0.27, 0.54)	0.07
C → D	0.40 (0.19, 0.65)	0.13	0.31 (0.20, 0.45)	0.07
D → A	0.01 (0.00, 0.09)	0.01	0.01 (0.00, 0.05)	0.01
D → B	0.01 (0.00, 0.09)	0.01	0.07 (0.04, 0.13)	0.02
D → C	0.10 (0.05, 0.19)	0.03	0.19 (0.13, 0.26)	0.03

Figures

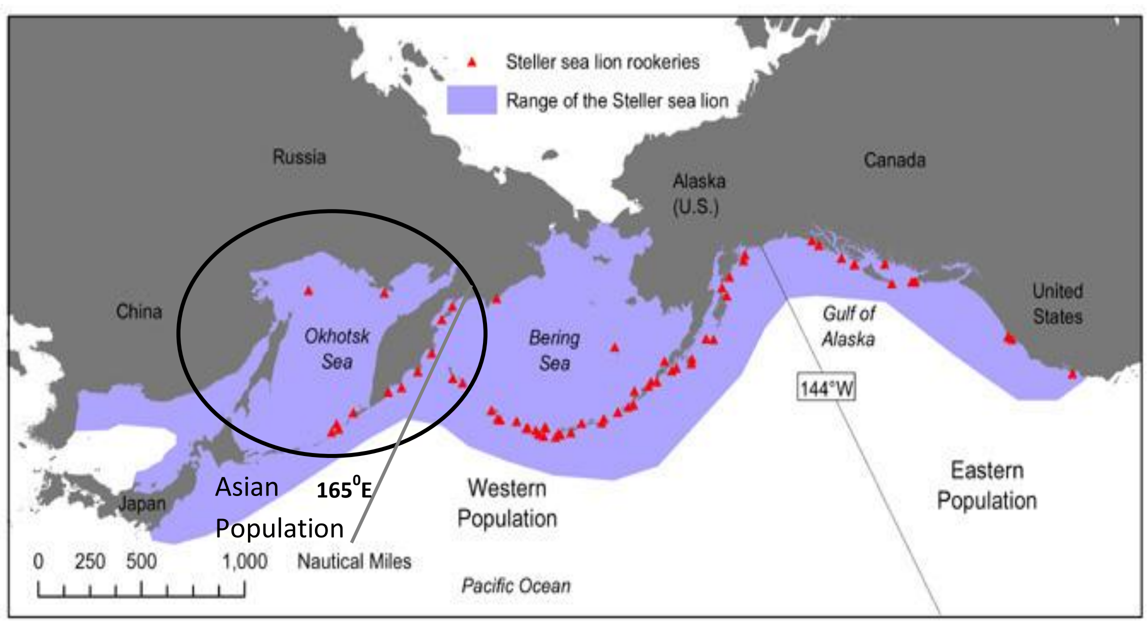

Figure 1.1. Map of Steller sea lion range and rookeries. Circle shows Russian Far East region study area. Adapted from NMFS, Alaska Fisheries Science Center (www.afsc.noaa.gov/stellers/range.htm) and Phillips et al. 2011.

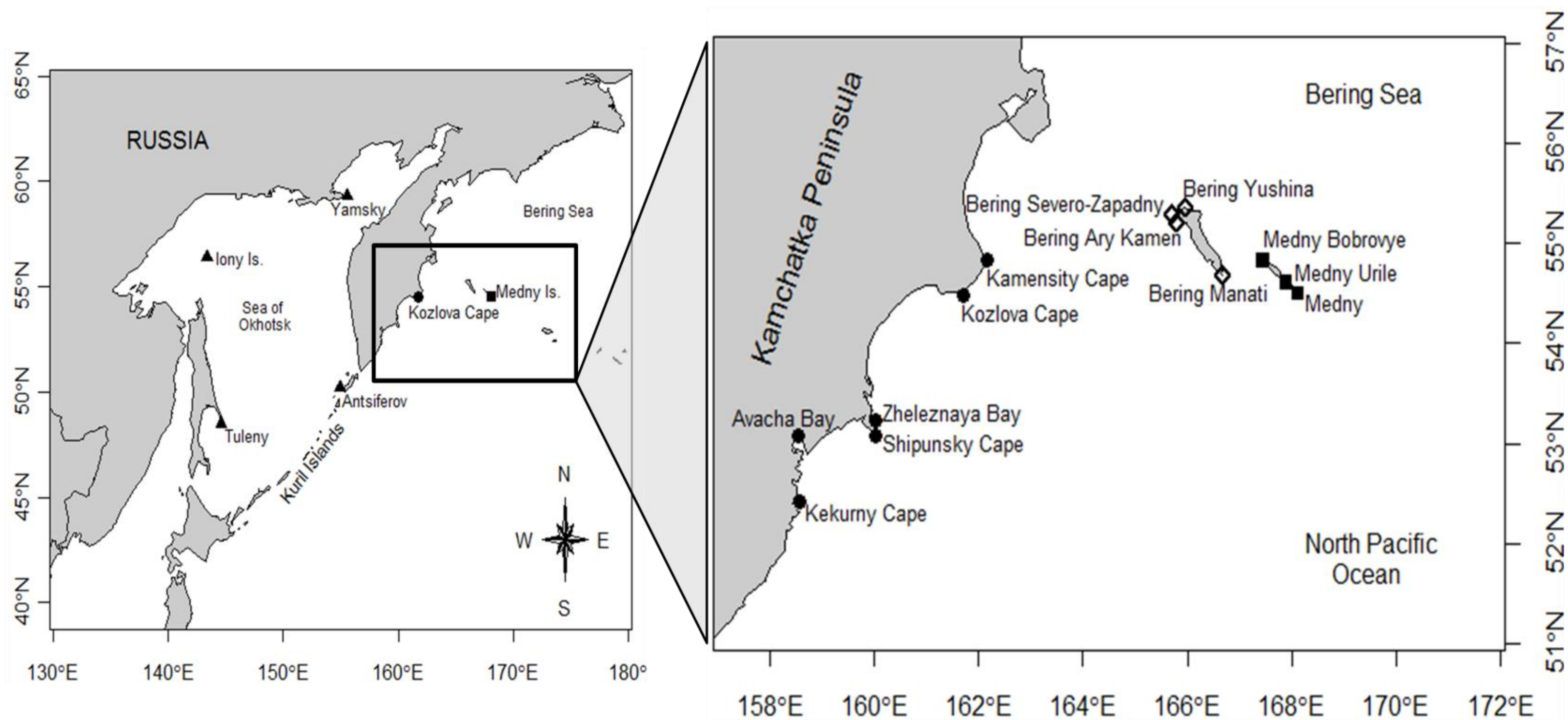

Figure 1.2. Map showing all 17 resighting locations. Triangles represent stratum A with natal rookeries Iony Is. and Yamsky, circles represent stratum B with natal rookery Kozlova Cape, open diamonds represent stratum C with no natal rookery, and squares represent stratum D with Medny being the natal rookery.

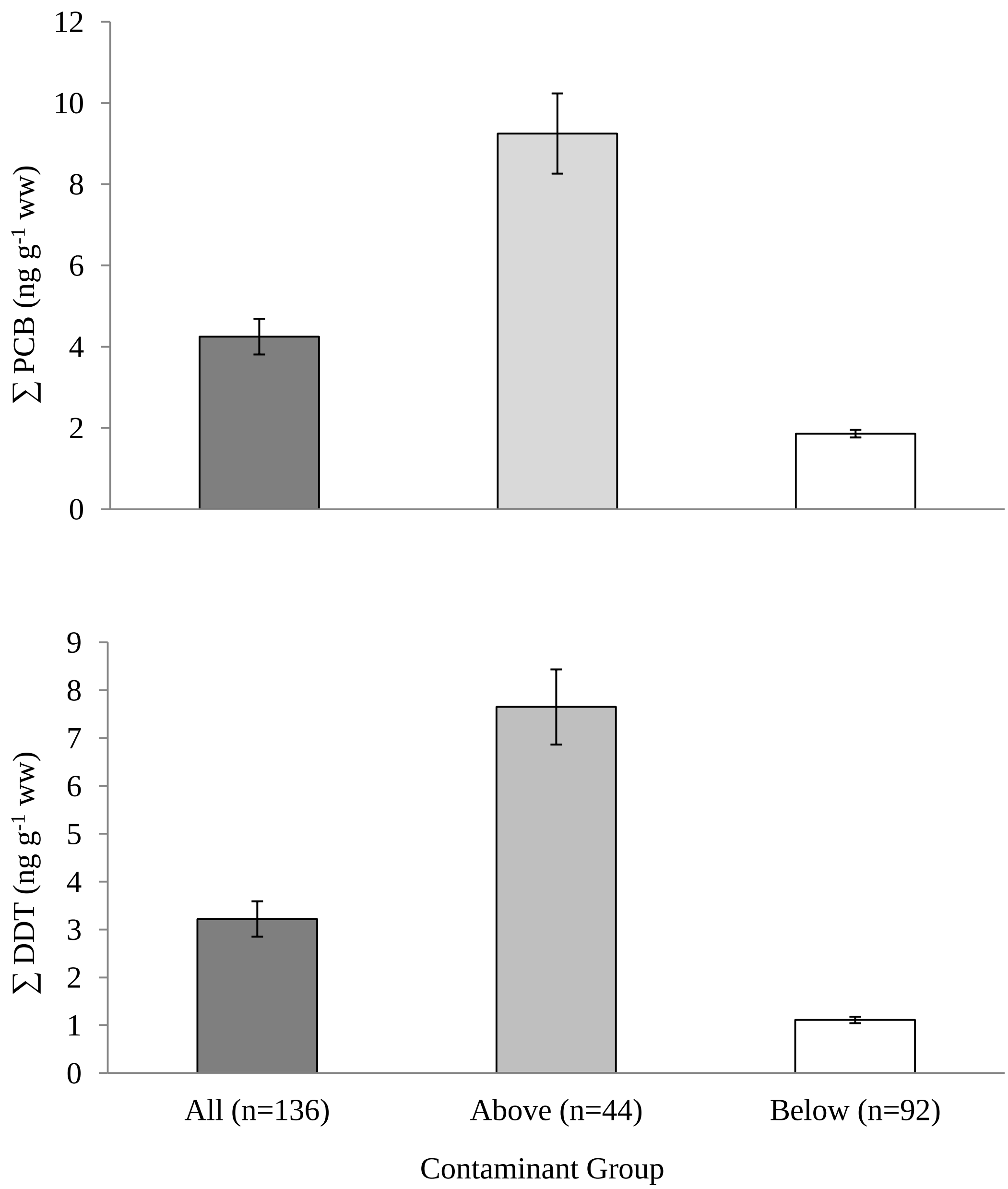

Figure 1.3. The average whole blood OC concentrations for all hot-branded SSL (n = 136) and for those in the above (n = 44) and below (n = 92) mean concentration groups, including standard error bars. The top panel shows the values for ∑PCB and the bottom panel shows the values for ∑DDT.

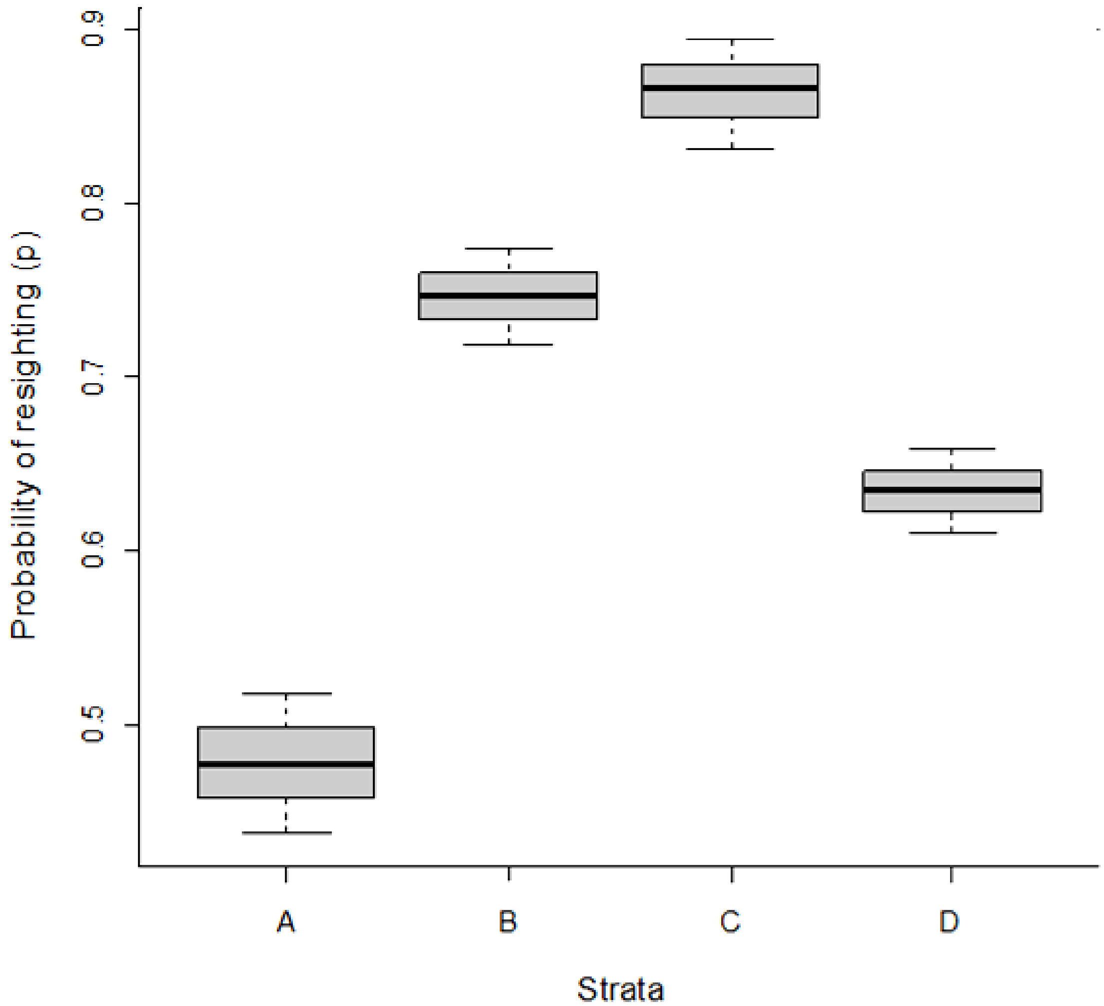

Figure 1.4. Estimates for the probability of resighting (p), using model ϕ(a,s,c), p(s,eff), Ψ(a,c), including 95% confidence intervals (dashed vertical lines) at each stratum averaged (horizontal black line in each boxplot) from 2003 – 2011. Strata: A (Iony Island and Yamsky), B (Kozlova Cape), C (Bering Island), and D (Medny Island).

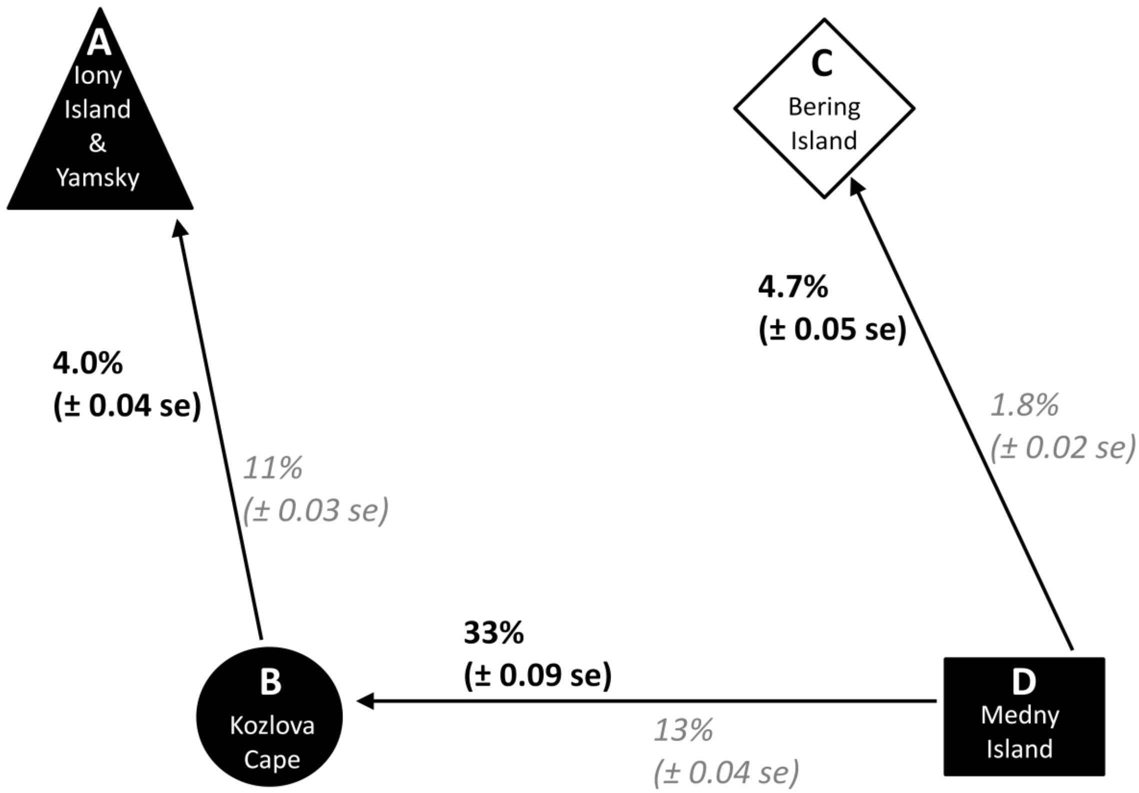

Figure 1.5. Selected movement probabilities (Ψ) for age group 0 using model ϕ(a,s,c), p(s,eff), Ψ(a,c). Results for SSL above (black text) and below (italic gray text) mean OC concentration group, including standard error (in parentheses) are shown.

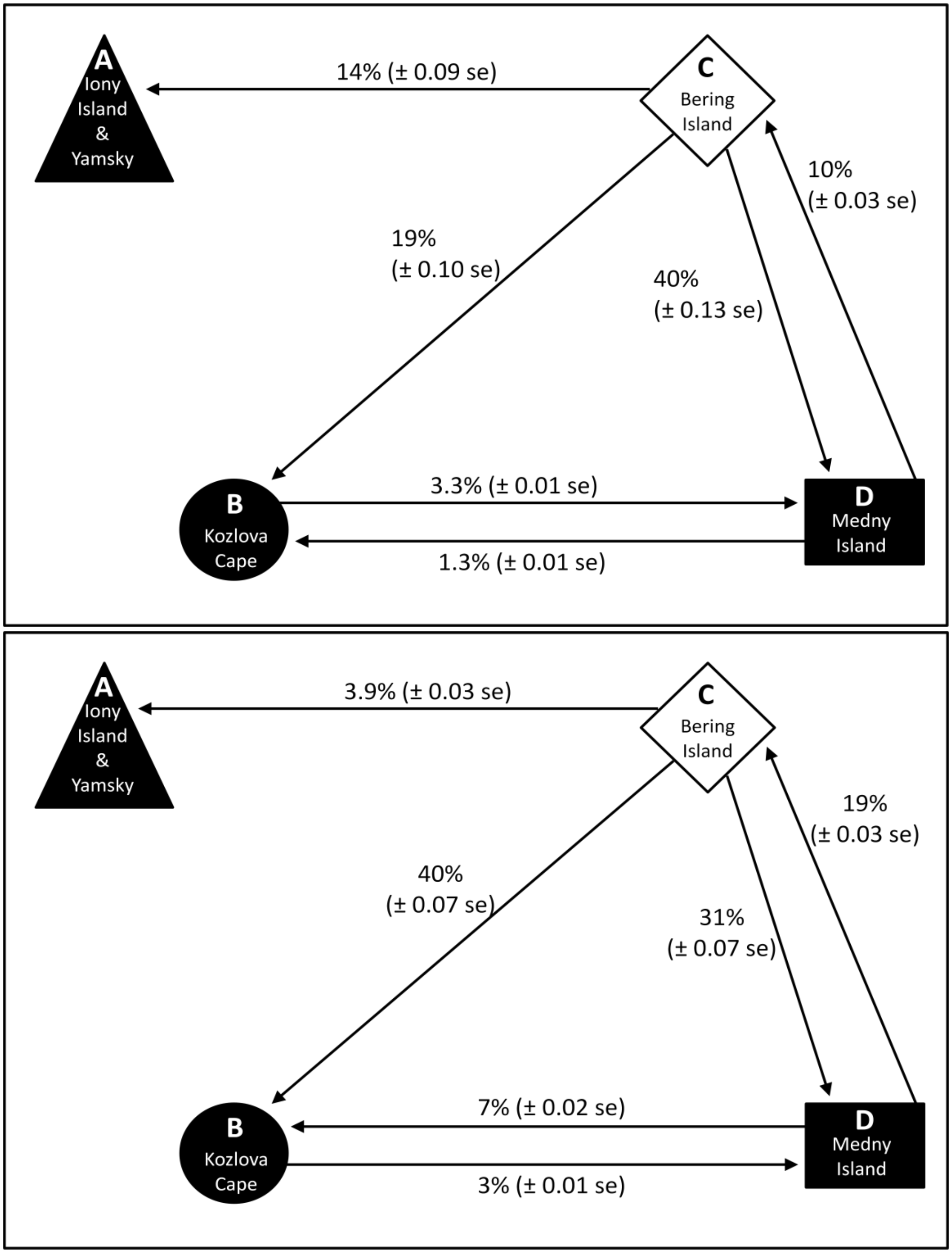

Figure 1.6. Selected movement probabilities (Ψ) for age group 1-9 (1+) using model ϕ(a,s,c), p(s,eff), Ψ(a,c), for above (top panel) and below (bottom panel) mean OC concentration groups with standard error (in parentheses).

Chapter 2: Concentrations of post-natal organohalogen contaminants and associations with female reproductive success in western Steller sea lions (*Eumetopias jubatus*)

Abstract

The western stock of Steller sea lions has been listed as an endangered species under the U.S. ESA since 1997 and has failed to recover throughout its range. There are numerous hypotheses as to the causes for their lack of recovery, one of which is exposure to anthropogenic contaminants. Persistent organohalogen contaminants (OCs), such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), polybrominated diphenylethers (PBDEs), and hexachlorobenzene (HCB), have not been ruled out as a potential cause for the lack of recovery and are associated with negative biological effects in other marine mammal species. The objective of this study was to determine the relationship between OCs and reproductive parameters using resighting histories from 128 female SSL pups hot-branded from 2001 – 2007 at nine rookeries in the Russian Far East. Reproductive success and age at first reproduction were analyzed using mixed effects models and linear regression, respectively. The age at first reproduction was significant at five of nine rookeries, suggesting that at some rookeries female SSL are having pups at older ages. Of the females hot-branded from 2001 – 2007, 41 – 48% had OC concentrations above thresholds suggested to have harmful biological

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effects. However, post-natal concentrations of OCs were not significant variables for predicting reproductive success. Similarly, the mean concentrations of ∑PCB, ∑DDT, and ∑PBDE were not significantly different between female SSL that were observed to have pups or not, indicating that these reproductive parameters were affected more by natal rookery than by OCs.

Introduction

Steller sea lions (*Eumetopias jubatus*) range from California's Channel Islands to northern Japan, with the western Steller sea lion (SSL) Distinct Population Segment occurring west of 144° longitude, which has been listed as endangered since 1997 under the Endangered Species Act (62 U.S. Federal Register 24345). The population has declined by approximately 80% since the 1970's (Holmes et al. 2007; Sease et al. 2001; Calkins et al. 1999) and researchers have commented on the importance of investigating contaminants as contributing factors (Wang et al. 2011; Atkinson et al. 2008; Myers et al. 2008; NMFS 2008; Barron et al. 2003). The influence of persistent organohalogen contaminants (OCs) in the habitats of SSL could affect vital rates, such as reproduction (Noonburg et al. 2010; Huntington 2009; Tanabe 2002).

Among the most abundant synthetic toxins measured in the tissues of SSL are OCs, such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), polybrominated diphenyl ethers (PBDEs), and hexachlorobenzene (HCB) (Wang et al. 2011; Myers et al. 2008; Barron et al. 2003). Both PCBs and DDTs belong to a group of contaminants referred to as "legacy" pollutants which are among the most prevalent pollutants measured in biota (Wang et al. 2012; Myers et al. 2008) and PBDEs belong to a group of “emerging” pollutants, used as flame retardants in electronics, plastics, and textiles (de Boer et al. 1998; EPA). Halogenated contaminants have high lipophilicity (Becker 2000; Watanabe et al. 1999), resist metabolic and environmental breakdown (Becker 2000; Lee et al. 1996), undergo vertical transfer to offspring (Beckmen et al. 2003, 1999), and biomagnify or increase in concentration at higher trophic levels (Tanabe 2002; Watanabe et al. 1999).

The most common uses for PCBs have been for machinery lubricants and as coolant for electrical equipment, DDTs and HCBs were commonly used as agricultural insecticides; however, their use has been banned in most developed countries since the 1970s and 1980s (Lee et al. 1996). Although HCBs are no longer used in most agriculture systems, they may still be formed as a byproduct in the production of chemical solvents, such as in dyes and wood preservatives (EPA). These chemicals are insidious in the Arctic because of atmospheric and oceanic transport from Eurasia and tropical regions, some of which still utilize a suite of these chemicals (Aguilar et al. 2002; Bard 1999; Iwata et al. 1994, 1993). The transport of OCs to northern latitudes is promoted by atmospheric distillation and cold air sinks that permit these compounds to be deposited in North Pacific habitats occupied by SSL (Li et al. 2002; Bard 1999; Iwata et al. 1994, 1993). Both PCB and DDT levels, measured in archived marine mammal blubber, were at their highest concentrations during the 1970’s, while DDT levels declined to one thirtieth of the greatest measured concentrations by the 1990’s and PCB levels declined to approximately half of their 1970 levels by the 1980’s and 1990’s (Tanabe et al. 2002).

The years with the greatest levels of contaminants correspond with the overall reduction in SSL populations and subsequent listing on the US Endangered Species Act, although no definitive cause and effect has been established for this relationship.

Threshold concentrations at which harmful biological effects are expected to occur have been proposed for marine mammals, which were determined from dead and stranded marine mammals, not including SSL. These harmful effects can include decreased endocrine and immune system response (Ross et al. 1995), and cancer (Ylitalo et al. 2005). A suggested PCB threshold concentration of 11,000 ng g^{-1} lipid weight (lw) and a wet weight (ww) concentration of 440 ng g^{-1}, as measured in the blood or liver, has been proposed for marine mammals (Kannan et al. 2000). There is no corresponding biological threshold concentration identified for DDT contaminants in marine mammals (Kannan et al. 2004). However, a general threshold concentration of 1ppm (1ppm = 1000 ng g^{-1}) has been recommended for any organohalogen contaminant or persistent organic pollutant in any tissue for marine mammals as an indication of high risk for negative biological effects (Letcher et al. 2010).

Bering Sea SSL have been identified as a species with considerable accumulations of OCs (Myers et al. 2008). Myers et al. (2008) found that 29% of Russian pups and 12% of Alaska pups exceeded the 11,000 ng g^{-1} lw threshold for the sum of all quantified PCBs (∑PCB) proposed for seals by Kannan et al. (2000), as measured in whole blood and, although not directly comparable, the sum of all quantified DDT contaminants (∑DDT) also exceeded the same threshold. There were much greater concentrations of ∑PCB and ∑DDT reported by Lee et al. (1996) for SSL from Alaska and the Russian Bering Sea sampled from 1976 – 1978 than the concentrations reported by Myers et al. (2008). The ∑PCB for male SSL ranged from 5,700 to 41,000 ng g^{-1} lw and from 570 to 16,000 ng g^{-1} lw for female SSL; ∑DDT values ranged from 2,800 to 17,000 ng g^{-1} lw for male SSL and from 190 to 6,500 ng g^{-1} lw for female SSL measured in the blubber and liver (Lee et al. 1996). Although the methods for chemical analyses differed between the two studies the results demonstrate potentially harmful concentrations of OCs in SSL.

Male SSLs likely experience an increasing body burden of contaminants with age, whereas females tend to show increasing levels followed by a sharp decrease at five years of age, which corresponds to reproductive maturity and pregnancy (Lee et al. 1996; Pitcher and Calkins, 1981). It is estimated that approximately 80% of PCBs and 79% of DDTs of a female's contaminant load are transferred through lactation (Lee et al. 1996) and some SSL pups have been observed to suckle periodically for up to four years (Mamaev and Burkanov, 2004). Mobilization of lipids increases in response to physiological demands and 19% to 27% of maternal body fat, depending on species, is mobilized in sea lions and seals during lactation and are present in milk (Oftedal, 2000). Northern fur seal (*Callorhinus ursinus*) pups from primiparous dams had greater concentrations of OCs than did pups from multiparous dams, resulting in decreased immune system response, putting neonates at greater risk of morbidity and mortality (Beckmen et al. 2003, 1999). Adult male harbor seals (*Phoca vitulina*) had higher mean concentrations of OCs than did females, and nursing dams may retain the more toxic non-*ortho* PCBs, thereby attenuating some of the toxic effects experienced by pups (Wang et al. 2007a,b). Galapagos sea lion (*Zalophus wollebaeki*) pups have concentrations (mean, 525 ng g^{-1}, lw) of ∑DDT, known to cause anti-androgenic effects in other vertebrates (Alava et al. 2011).

The objective of the present study was to use OC measurements and resighting data from hot-branded SSL pups from the Russian Far East to investigate the relationship between OCs and female reproduction. The null hypotheses tested were: (1) the age at first reproduction is not associated with post-natal contaminant load, and (2) reproductive success is not associated with post-natal contaminant load. To test these hypotheses, 128 blood samples were collected from 2001 - 2007 at nine rookeries from free-ranging Steller sea lion pups in the Russian Far East and were analyzed for a suite of PCB's, DDT's, PBDE's, and HCB. Using resighting data the potential effects of OCs on female reproductive traits in this region were estimated.

Methods

Sample Collection

Field efforts have been conducted to individually mark SSL pups, to collect blood samples for health assessments, and to conduct resighting surveys at SSL rookeries in the Russian Far East since 2001. The procedure for individually marking SSL pups follows the methods of Merrick et al. (1996) for applying unique alphanumeric hot-brands. The method of hot-branding pinnipeds has been shown to be the most efficient way to apply a long-lasting individually identifiable mark that is visible from long distances and causes minimal harm to the animal (Hastings et al. 2009; Merrick et al. 1996). Hot-brands on

SSL have been reported to remain legible for at least seven years (Merrick et al. 1996) and have been routinely seen 10 – 14 years post-branding. Prior to hot-branding, individuals are anesthetized following the protocol of Hastings et al. (2009) and Heath et al. (1996) with the use of mobile isoflurane gas as an anesthetic.

The study area included a segment of the endangered western and the proposed Asian stock. For the purposes of this study, to investigate effects on reproduction, our data included only female pups hot-branded at nine rookeries (Figure 2.1). Blood samples were collected (n = 128) from late June to early July from hot-branded free-ranging SSL pups approximately three weeks old (Myers et al. 2008).

Resighting histories of 62 hot-branded SSL pups born in 2002 were obtained by photo-documentation during June – December 2003 – 2011. Resighting effort was conducted at major rookeries and haulout sites across the Russian Far East during boat based surveys. Branded SSL were photographed from small boats, land-based vantage points, and field camps at Kozlova Cape, Medny Island, Tuleny, and Antsiferov rookeries. Kozlova Cape and Medny Island used remote video and still camera systems maintained by personnel at nearby field camps. The remote monitoring systems at these locations were described in a previously published study (Burdin et al. 2009). Only those resightings accompanied by a confirmed image of the brand were used as confirmed animal sightings. All samples and corresponding data, collected during the 2002 field season, were maintained by North Pacific Wildlife Consulting in collaboration with the National Marine Mammal Laboratory (Myers et al. 2008).

The analyses of OCs were measured using two different laboratory techniques and will therefore not be directly compared, but separately discussed. The initial study was completed in 2008 using a high performance liquid chromatography technique (Ylitalo et al. 2005; Krahn et al. 1994). This technique was developed as a rapid screening method used to quantify pollutant levels in whole blood samples from 136 SSL pups from the Russian Far East (Myers et al. 2008). These SSL were branded in 2002, of which 62 were female and only 25 were observed subsequent to reaching four years of age, which is the earliest age they would be expected to have pups (Table 2.1). These samples were collected from four Russian Far East rookeries: Iony Island (female n=12), Kozlova Cape (female n=17), Medny Island (female n=20), and Yamsky (female n=13).

An additional 103 blood serum samples were analyzed from female SSL pups, hot-branded from 2001 – 2007, that were resighted at least once since reaching maturity (Table 2.2). These data were collected from nine Russian Far East rookeries: Iony Island (female n=12), Kozlova Cape (female n=3), Medny Island (female n=5), and Yamsky (female n=10), Srednego (female n=8), Brat Chirpoyev (female n=16), Lovushki (female n=14), Raykoke (female n=12), and Antsiferov (female n=24).

Chemical Analyses

Quantification of 15 PCBs (77, 101, 105, 118, 126, 128, 138, 153, 156, 157, 169, 170/194, 180, 189) and six chlorinated contaminants (o,p'-DDD, p,p'-DDD, p,p'-DDE, o,p'-DDT, p,p'-DDT, hexachlorobenzene), were measured using high-performance liquid chromatography/photodiode array, these results were previously published in Myers et al.

(2008). The total concentration of ∑PCBs was calculated by summing all 15 PCB congener concentrations and similarly performed for ∑DDT. Total lipid quantities in each whole blood sample were measured by thin layer chromatography with flame ionization detection, previously reported in Myers et al. (2008).

Additional analyses were conducted in 2012 using gas chromatography – mass spectrometry (GC-MS) to quantify OC congeners. From each blood serum sample we quantified: 14 PCB congeners (77, 101, 126, 105, 118, 169, 128, 189, 153, 138, 157, 170, 156, 180), 6 DDT metabolites (o,p’-DDD, p,p’-DDD, o,p’-DDE , p,p’-DDE, o,p’-DDT, p,p’-DDT), HCB, and 8 PBDE congeners (3, 15, 28, 47, 99, 154, 153, 183). Sample cleanup was performed following the procedure of Qu et al. (2007) with modifications. There were 5 µl (10 ppm) of each surrogate standard: 2, 4, 5, 6-tetrachloro-mxylene (TCmX) and decachlorobiphenyl (PCB-209), added to the blood serum samples. The surrogate standards were compounds similar to the OCs being measured and were added to the blood serum samples at a known concentration in order to determine extraction efficiency or recovery rates. The average recoveries for the surrogate standard TCmX was 60 ± 8% and 85 ± 10% for PCB-209 and were used as correction factors when quantifying contaminant levels. The range of recoveries for the OCs was from 72% to 110% with a limit of detection from 1 – 10 pg g^{-1}. Following lipid cleanup, using n-hexane and H_2SO_4, the organic solvent was reduced to approximately 1 mL under nitrogen flow then cleaned again with hexane (2 µl); this solution was then completely dried under nitrogen flow before adding 50 µL of hexane. Prior to GC-MS analysis pentachloronitrobenzene was added as the internal standard. The internal standard had a chemical structure similar to the OCs being quantified and was added to the blood serum samples at a known concentration and used for the calibration of the analyzed OCs. Quality control for GC-MS was done with hexane blanks and a hexane acetone blank for tip cleaning; the blanks were run between every two true samples.

Statistical Analyses

Differences in reproductive success relative to ∑PCB, ∑DDT, ∑PBDE, age, and natal rookery were tested using generalized linear mixed effects models fit with an autoregressive correlation function to account for a repeated measures design. Reproductive success was defined using binary coding (1 = successful, 0 = unsuccessful). Differences in age at first reproduction were also tested against ∑PCB, ∑DDT, ∑PBDE, and natal rookery using linear models. Age at first reproduction was quantified as the first event when a female was observed to have a pup and was at least four years of age. Natal rookery was used as a categorical variable containing all natal rookery locations for female SSL pups. For the data set of females sampled from 2001 – 2007 (n = 103) age at first reproduction was also modeled separately for females that had pups (n = 59) and those that did not have pups (n = 44). This procedure was also performed for females sampled in 2002 (n = 25), but had small sample sizes for females with pups (n = 17) and females without pups (n = 8). Because not all branded female SSL could be observed continuously, reproductive success and age at first reproduction are minimum estimates, but nonetheless our best estimates based on observational data. Correlations between predictor variables were examined using Pearson's product-moment correlation coefficients. Statistical analyses were implemented using R version 2.13.0 (R Development Core Team, 2011). A significance level α of 0.05 was used for all statistical significance tests.

Model comparison and selection were performed using AICc (Akaike Information Criterion, second order) to account for small sample size, when $n/k < 40$, where n is sample size and k is the estimated parameters (Burnham and Anderson, 2002). When model AICc values were close, then model parsimony and AICc differences (Δ) were considered. Model support is based on the Kullback-Leibler distance between models represented as ΔAICc, where the larger the Δ the less support there is that the model adequately explains variation in the data (Burnham and Anderson, 2002). A Δ range from 0 – 2 provides strong support for the model being the best model, Δ 2 – 4 indicates weak support for the model not being best model, Δ 4 – 7 indicates moderate support for the model not being the best model. If the model considered has a Δ 7 – 10 then it provides strong evidence that the model is not the best model and a Δ > 10 very strongly indicates that the model is not the best model and fails to adequately explain a considerable amount of variability (Burnham and Anderson, 2002).

Results

Female SSL (n=25) sampled in 2002

Mean whole blood ∑PCB concentrations, with standard error, for female SSL not resighted (n = 31) was 5.65 ± 1.06 ng g^{-1} ww, for female SSL resighted without a pup (n = 14) was 3.00 ± 0.60 ng g^{-1} ww, and for SSL resighted with a pup (n = 17) was 4.63 ± 1.26 ng g^{-1} ww (Figure 2.2). Mean whole blood ∑DDT concentrations, with standard error, for female SSL not resighted was 4.90 ± 1.19 ng g^{-1} ww, for female SSL resighted without a pup was 2.74 ± 0.79 ng g^{-1} ww, and for female SSL resighted with a pup was 3.24 ± 0.88 ng g^{-1} ww (Figure 2.2). There were no statistically significant differences ($p > 0.05$) between groups. The Pearson's product-moment correlation coefficient between ∑PCB and ∑DDT ($r = 0.95$, $p < 0.05$), indicated that it is not statistically appropriate to include both in the same model for the reason of multicollinearity.

Reproductive Success

Mixed-effects models indicated that ∑PCB and ∑DDT were not significant variables for predicting reproductive success. The general ∑PCB model 9, $Pbi = \beta_0 + \beta_1 PCB + \beta_2 NR + \beta_3 Wt + \varepsilon$, had an AICc value of 175.93 and model 6 excluding ∑PCB, $Pbi = \beta_0 + \beta_1 NR + \beta_2 Wt + \varepsilon$, had an AICc value of 174.74 (Table 2.3a), where PCB is ∑PCB concentrations, NR is natal rookery, and Wt is birth weight of each female. The AICc criterion therefore suggests that both models explain the data about the same and that a higher AICc value for the model with ∑PCB suggests that a PCB effect was not detected. Similarly for ∑DDT model 10, $Pbi = \beta_0 + \beta_1 DDT + \beta_2 NR + \beta_3 Wt + \varepsilon$, which had an AICc value of 176.33 compared to that for model 6, also suggesting that a DDT effect was not detected (Table 2.3a). The same qualitative results occurred for the other models in Table 2.3a, which omitted one or both of the variables Wt and NR. In fact, the most parsimonious model 1 simply had the random effects term (Appendix Table 2.1).

Female SSL at Kozlova Cape were reproductively more successful ($p < 0.05$) when compared to other rookeries, but only when Wt and OCs were also included as predictor variables, as in the general models for ∑PCB and ∑DDT (Table 2.3a,b). An analysis of variance of Kozlova Cape female pups indicates that the two smallest individuals (25.5 and 23.5 kilograms) had greater ∑PCB and ∑DDT concentrations ($p < 0.05$) when compared to other Kozlova Cape female pups that successfully reproduced during the same period. Weight was not statistically significant ($p > 0.05$) in model 3, $Pbi = \beta_0 + \beta_2 Wt$, suggesting no effect of female pup birth weight (Appendix Table 2.1). When compared to other rookeries there was significantly ($p < 0.05$) lower reproductive success for Iony Island in model 5, $Pbi = \beta_0 + \beta_2 NR$ (Table 2.3b).

Age at First Reproduction

Similar to the mixed effects models for reproductive success, linear regression models were used to identify relationships of age at first reproduction with OCs, natal rookery, and weight, as described above (Table 2.4a). There were no significant relationships between age at first reproduction and ∑PCB or ∑DDT (models 13 and 14 in Table 2.4b). A minor significant interaction effect was found between Kozlova Cape and weight ($p = 0.05$) (model 12 in Table 2.4b), but only when ∑PCB was included in the model; Age = $\beta_0 + \beta_1$PCB + β_2NR + β_3Wt + β_4NR*Wt + ε, and similarly for the ∑DDT model, Age = $\beta_0 + \beta_1$DDT + β_2NR + β_3Wt + β_4 NR*Wt + ε (model 15 in Table 2.4b). The most parsimonious model 1 in Table 2.4a only contained a natal rookery effect, and in all models that contained one or both variables NR, Wt, and their interaction; neither PCB nor DDT lowered the AICc, suggesting an inability to detect and OC effect (Appendix 2.2).

Female SSL (n=103) sampled from 2001 – 2007

There were no statistically significant differences for ∑PCB, ∑DDT, and ∑PBDE between females that did produce pups (n = 59) and those that did not (n = 44). Mean blood serum ∑PCB concentrations, with standard error, were 4.79 ± 0.45 ng mg^{-1} lw and 3.93 ± 0.35 ng mg^{-1} lw for those without and with pups, respectively, and were not statistically significant (ANOVA, $p > 0.05$). Mean blood serum ∑DDT concentrations were 1.97 ± 0.31 ng mg^{-1} lw and 2.18 ± 0.26 ng mg^{-1} lw for those without and with pups, respectively, and were not statistically significant (ANOVA, $p > 0.05$). Mean blood serum ∑PBDE concentrations were 0.42 ± 0.04 ng mg^{-1} lw and 0.39 ± 0.03 ng mg^{-1} lw for those without and with pups, respectively, and were not statistically significant (ANOVA, $p > 0.05$). The overall mean serum concentrations for ∑PCB, with standard error, were 4.30 ± 0.28 ng mg^{-1} lw, for ∑DDT it was 2.09 ± 0.20 ng mg^{-1} lw, and for ∑PBDE it was 0.40 ± 0.02 ng mg^{-1} lw. The range of OC concentrations for all female SSL varied widely by rookery location with no clear trend for ∑PCB (Figure 2.3) or ∑DDT (upper panel in Figure 2.4), as indicated by the spread of means, and ∑PBDE had a narrower range of means compared to OC concentrations at other rookeries (lower panel in Figure 2.4). The Pearson's product-moment correlation coefficients between ∑PCB and ∑DDT were $r = 0.65$ ($p < 0.05$), $r = 0.77$ ($p < 0.05$) for ∑PCB and ∑PBDE, and $r = 0.70$ ($p < 0.05$) ∑PBDE and ∑DDT, so it is not statistically appropriate to include combinations of these OCs in the same model because of multicollinearity.

Reproductive Success

The best predictor variable for reproductive success in the mixed effect models was natal rookery (model 1 in Table 2.5a). The general models used to test the effects of natal rookery (NR) and OCs on reproductive success were: $Pbi = \beta_0 + \beta_1 PCB + \beta_2 NR + \varepsilon$, $Pbi = \beta_0 + \beta_1 DDT + \beta_2 NR + \varepsilon$, and $Pbi = \beta_0 + \beta_1 PBDE + \beta_2 NR + \varepsilon$ (models 2 - 4 in Table 2.5b). There was no OC effect detected in these or more complex models (Table 2.5a and Appendix Table 2.3).

Reproductive success was significantly ($p < 0.05$) lower at Iony Island and Srednego in all models tested both with and without OCs as independent variables (Table 2.5b). Reproductive success at Antsiferov was significant in models 1 and 4 (Table 2.5a), indicating that reproduction at this location may be negatively affected by OCs. Including OCs did not produce lower AICc's or statistically significant coefficients; nonetheless, Antsiferov had a greater overall mean ∑DDT concentration (2.41 ± 0.45 ng mg^{-1}), where 13 out of 24 (52%) females were observed with pups, compared to ∑DDT at Iony (1.47 ± 0.60 ng mg^{-1}) and Srednego (1.72 ± 0.63 ng mg^{-1}).

Age at First Reproduction

Linear regression models of age at first reproduction indicated that there was a significant negative interaction between natal rookery with ∑DDT, ∑PBDE, and ∑PCB (models 1, 2 and 7 in Table 2.6a, b). The age at first reproduction was significant ($p < 0.05$) at natal rookeries Antsiferov, Brat Chirpoyev, Iony Island, Srednego, and Yamsky, which suggests that female SSL are having pups at older ages when compared to all other rookeries using a model of the form: $Age = \beta_0 + \beta_1 PCB + \beta_2 NR + \varepsilon$ (Table 2.6a,b). A negative ∑PCB effect was detected for age at first reproduction for the same rookeries, except Antsiferov, when allowing for an interaction effect between ∑PCB and natal rookery, $Age = \beta_0 + \beta_1 PCB + \beta_2 NR + \beta_3 PCB * NR + \varepsilon$. Similarly for ∑PBDE, the natal rookeries Antsiferov, Brat Chirpoyev, Iony Island, Srednego, and Yamsky were estimated to have pups at older ages when compared to other rookeries using the model: $Age = \beta_0 + \beta_1 PBDE + \beta_2 NR + \varepsilon$. There was a negative ∑PBDE effect detected at Antsiferov, Brat Chirpoyev, and Srednego rookeries when including an interaction effect between ∑PBDE and natal rookery using a model of the form: $Age = \beta_0 + \beta_1 PBDE + \beta_2 NR + \beta_3 PBDE*NR + \varepsilon$, suggesting a harmful ∑PBDE effect on age at first reproduction (Table 2.6b).

The best ∑DDT model, $Age = \beta_0 + \beta_1 DDT + \beta_2 NR + \varepsilon$, showed that the natal rookeries Antsiferov, Iony Island, Srednego, and Yamsky were having pups at older ages when compared to all other rookeries (model 6 in Table 2.6b). Alternate ∑DDT models failed to adequately explain additional model variability based AICc values (Table 2.6a). Models including only OCs (models 8 – 10 in Table 2.6a, b), with the exception of

∑PCB, did show a negative relationship to age at first reproduction; however, model fit was weak, with a model $R^2 < 0.05$, indicating that variation in age at first reproduction was not well explained by OCs .

The two most parsimonious models 1 and 2, from Table 2.6a, included interactions of PCB and PBDE with natal rookery, suggesting there were different effects of these OC's at different rookeries. The AICc values for models 5 and 3 without the interaction term were about 2.5 and 8.5 units higher than models 1 and 2, respectively, suggesting that these interactions are real (Table 2.6a). Therefore, age at first reproduction must be examined at the rookery level.

For females that were seen with pups, only ∑PCB was significantly correlated to age at first reproduction (Appendix Table 2.4). Model fit, however, indicated that this relationship explained only a small part of the total variation with a model $R^2 = 0.06$. There was no OC effect detected in interactive models 9 - 11, except for Iony Island rookery, which was the only location with a significant and negative interaction effect estimated for ∑PCB and ∑PBDE (Table 2.7a, b), with an average age at first reproduction of 6 ± 0.71. Age at first reproduction for Antsiferov and Yamsky rookeries was significant ($p < 0.05$) in additive models including OCs and natal rookery (models 1, 3, and 4 in Table 2.7 a, b) indicating an older age at first reproduction for female SSL at that rookery (Table 2.7b). The mean age at first reproduction for females with pups for all rookeries was 5.20 ± 0.14 years and the mean for Antsiferov and Yamsky was 5.15 ± 0.25 and 7.00 ± 0.55, respectively. The most parsimonious model 1 in Table 2.7a included both natal rookery and ∑PCB variables with an AICc of -14.34, and model 2 which excludes the ∑PCB variable has an AICc of -13.77; therefore, the AICc criterion suggests both models explain the data nearly the same. The difference in AICc values between models 1 – 4 is less than 4 units, suggesting those models are nearly equivalent and an OC effect was not detected.

There was no ∑PCB effect detected ($p > 0.05$) for females that were never seen with pups, but there was a ∑PBDE and ∑DDT effect ($p < 0.05$) on age at first reproduction (Appendix Table 2.5). Although, model fit was weak with an $R^2 \leq 0.12$, suggesting these relationships did not explain a majority of the model variability. The selected models included: $Age = \beta_0 + \beta_1 PCB + \beta_2 NR + \beta_3 PCB * NR + \varepsilon$; $Age = \beta_0 + \beta_1 DDT + \beta_2 NR + \beta_3 DDT * NR + \varepsilon$; and $Age = \beta_0 + \beta_1 PBDE + \beta_2 NR + \beta_3 PBDE * NR + \varepsilon$ (Table 2.8a). Common to these models was a significant ($p < 0.05$) but negative interaction effect between all OCs and Brat Chirpoyev, suggesting a potential negative OC effect on age at first reproduction at this rookery (Table 2.8b). The rookeries at Srednego and Yamsky were significant ($p < 0.05$) in ∑PCB model 11 in Table 2.8a, indicating a potentially negative effect of ∑PCB, resulting in estimates of an older age at first reproduction when compared to other rookeries. Antsiferov was significant in each model from Table 2.8a including the NR variable, except model 6, indicating that females at this rookery tended to be older than at other rookeries and that there are more females resighted without pups when compared to other rookeries (Table 2.2). The estimates for the interaction effect between OCs and natal rookery showed a significant ($p < 0.05$) negative relationship between Iony Island, Srednego, and Yamsky with ∑PCB and for

Srednego with ∑PBDE on age at first reproduction, suggesting a potentially negative OC effect at those rookeries (Table 2.8b). Iony Island had significant postive estimates in all additive models including OCs and natal rookery, suggesting reproduction occurred at older ages compared to other rookeries (models 3, 8, and 10 in Appendix Table 2.8b).

Discussion

Reference to established OC threshold values proposed by Kannan et al. (2000) and Letcher et al. (2010) helps to identify rookeries of concern. This was especially the case for the 2001 – 2007 data set where 41% - 48% of SSL female's mean OC concentrations were well above threshold values proposed by Letcher et al. (2010). The temporal variation in blood OCs could have been dependent on season and metabolism of blubber stores, so concentrations may have changed more quickly in blood whereas blubber concentrations may have changed over longer periods; nevertheless, the use of whole blood to accurately quantify OCs has been established in captive SSL (Myers and Atkinson, 2012). The lack of significance for OCs and trends in poor reproductive performance suggests that there is no relationship or that the biological parameters being measured may be insufficient to determine the negative biological effects of OCs.

The data from 2002 suggest that females with concentrations of OCs well above the group average are capable of surviving and reproducing, but does not yield information on pup survival. Female SSL pups had higher OC concentrations compared to males and Myers et al. (2008) suggested that larger pups have more blood volume than small pups, thereby reducing concentrations of OCs in blood. The pups of primiparous dams born with high OC concentrations (Beckmen et al. 2003, 1999) could receive a lethal dose of OCs during gestation and suckling, leading to aborted fetuses or moribund pups. These responses would clearly lower pup survival, but the data needed to establish these connections does not exist. The data and analyses presented in this paper are an initial attempt to understand how OCs may affect SSL reproduction, which may provide insight into the stock's lack of recovery. The reproductive parameters considered here were estimated using resighting data and therefore depend on the ability to observe those reproductive events. Because it is reasonable to assume that a SSL could give birth to a pup in a location outside of our survey area, our data are limited to minimum estimates from verified observations and the knowledge that site fidelity is practiced in this species (NMFS 2010; Trites et al. 2006; Raum-Suryan et al. 2002). The ability to continue resighting efforts on SSL that already have a known history is critical to understanding the complexities of species specific vital rates.

Female SSL (n=25) sampled in 2002

The average concentrations of ∑PCB and ∑DDT tended to be higher for SSL that had successfully reproduced (∑PCB, 4.63 ± 1.26; ∑DDT, 3.24 ± 0.88) than those that had not (∑PCB, 3.00 ± 0.60; ∑DDT, 2.74 ± 0.79). However, there was no significant difference ($p > 0.05$) between group means, tested using Tukey's Honest Significant Difference method. Female SSL on Medny Island that successfully reproduced had slightly higher concentrations for all OCs when compared to those not resighted, but the difference was not significant (F-test, $p > 0.05$). There appears to be no reliable trend in the data suggesting that contaminants have a consistently negative effect on female reproduction.

The natal rookery at Kozlova Cape was the last location to be sampled in this study, resulting in pups potentially nursing for up to two days longer than at the previously sampled rookery; therefore being slightly larger than pups at the other three rookeries. Kozlova Cape had the most females resighted with pups (n = 9), compared to the next highest at Medny Island (n = 5), but had a lower average for total reproductive success, 2.22 ± 0.46 at Kozlova Cape compared to 3.22 ± 0.66 pups per female at Medny Island. The two smallest females at Kozlova Cape also had the greatest contaminant concentrations for both ∑PCB (17 and 19 ng g^{-1} ww) and ∑DDT (11 and 13 ng g^{-1} ww). Both of these females successfully reproduced, one had three pups with the first at four years of age and the other had one pup with the first at five years of age. These OC concentrations are lower or similar to mean concentrations found in first born northern fur seal pups and neonates that had measurably compromised immune systems (∑PCB mean concentration of 16.23 to 22.84 ng g^{-1} ww, respectively, and ∑DDT mean concentrations of 6.17 to 13.53 ng g^{-1}) (Beckmen et al. 2003). However, that study quantified 11 PCB congeners and one DDT metabolite compared to this study of 15 PCB congeners, six DDT metabolites, and HCB. These results could imply that at wet weight concentrations of ∑PCB ranging from 17.0 – 19.0 ng g^{-1} and concentrations of ∑DDT ranging from 11.0 – 13.0 ng g^{-1} may not inhibit a female's ability to produce offspring, but may enhance it through estrogenic effects of OCs (Wójtowicz et al. 2007; Di Lorenzo 2002; Katzenellenbogen et al. 1995; Kelce et al. 1995; Colborn et al. 1993). However, first born pups from these females may be prone to morbidities due to weakened immune systems. The two female pups with the greatest concentrations of OCs that were never resighted after branding were from Iony Island and Yamsky rookeries and had the same ∑PCB concentration of 20.0 ng g^{-1} ww and ∑DDT concentrations of 25.0 and 26.0 ng g^{-1} ww, lending support to this supposition. However, this trend was not consistent throughout the data and there were females with OC concentrations below 1 ng g^{-1} ww that were also never resighted after 2002. Nonetheless, pups with higher concentrations of OCs, on average, tended to have lower resighting rates than their conspecifics with lower OCs as pups.

Female SSL (n=103) sampled from 2001 – 2007

Reproductive success showed no clear trend in being associated with varying OC concentrations, but rather appeared to be more affected by natal rookery. Reproductive success was lower at Iony Island and Srednego, such that only one of eight females was seen with a pup at Srednego and four of 12 females had pups at Iony Island. There were 13 of 24 females that successfully had pups at Antsiferov, which had lower concentrations of OCs compared to Srednego and higher concentrations when compared to Iony Island for females with pups (Figure 2.3, 2.4). However, there were no significant differences in OC concentrations between females with or without pups at Antsiferov (ANOVA, $p < 0.05$). The mean OC concentrations at Srednego tended to be high and Iony Island was in the lower range, but both locations had lower means than females at Medny Island. One problem in interpreting these results is the wide range of OC concentrations, particularly at Iony Island where three of eight females branded in 2004, which did not have pups, were among the most contaminated SSL females; however, females at Medny Island that did have pups had even greater concentrations than those that did not at Iony Island. Medny Island had the highest average concentrations for all rookeries and all females successfully had pups. The greatest overall concentrations for both ∑PBDE and ∑DDT for any rookery were from two females at Antsiferov, which successfully had pups at four and five years of age. Low reproductive success combined with relatively high OC concentrations likely resulted in the estimates of poor reproductive success for Iony Island and Srednego.

Age at first reproduction was not affected by OCs, such that OCs resulted in models with low R^2 values, but there were differences between ages at first reproduction between natal rookeries. The natal rookeries Antsiferov, Brat Chirpoyev, Iony Island, Srednego, and Yamsky all had females that appeared to be more reproductively active at older ages than all other rookeries. This result could partly be explained by resighting effort; at Iony Island and Yamsky resighting effort was often low or nonexistent for several years, but at Antsiferov, Brat Chirpoyev and Srednego resighting effort was high and consistent due to field camps conducting surveys throughout the breeding season. Interestingly these rookeries are at the northern extent (Iony Island and Yamsky) of the SSL range and southern end of the Kuril Islands (Brat Chirpoyev and Srednego), so there could possibly be environmental and biological factors, such as food, space availability, and other density dependent factors limiting successful pup production in young females. Conversely, Antsiferov rookery is located at the southern tip of the Kamchatka Peninsula in a region where the SSL population may be increasing (Burkanov and Loughlin, 2005), which could result in younger females having to wait a year or more to begin breeding on the main rookery. These same rookeries had significant negative correlations with age when an interaction term was included for ∑PCB, similarly with Brat Chirpoyev, Srednego and ∑PBDE. Because the models that only included OCs did not provide sufficient evidence of having negative effects on reproductive success or age at first reproduction, it's difficult to determine if there is indeed a rookery-specific effect with OCs. This is especially difficult to understand because the rookeries that stand out were not the most contaminated with the exception of Srednego being near the mean concentration of Medny Island, the most contaminated rookery, for ∑PCB. This is also the case with Antsiferov, which also had a very wide range of OC concentrations and the two most contaminated females for ∑PBDE and ∑DDT were still observed to have pups.

The research by Myers et al. (2008) found that there were rookery specific differences in the Russian Far East. In contrast to these contaminant data from 2001 – 2007, Myers et al. (2008) showed that Medny Island was the least contaminated Russian site while Kozlova Cape was the most contaminated with ∑PCB and Iony Island had the greatest concentrations of ∑DDT. The same trends of contamination were not observed in our study, which may reflect differences in sampling pups from primiparous or multiparous dams as well as temporal differences associated with season and OC distribution.

When the data were separated into female SSL that were observed with pups and those that were not, natal rookery was the best predictor, and OCs did not appear to have a negative effect on reproductive success or age at first reproduction. Similar to the models that included all females (n = 103), SSL at natal rookeries Brat Chirpoyev, Iony Island, Yamsky, and Srednego were having their first pups at older ages, but when an interaction term was included for OCs and natal rookery then age at first reproduction decreased with increasing OC concentrations. The same results were found for Brat Chirpoyev, Iony Island, and Srednego rookeries for SSL female's that did not successfully reproduce. The youngest pup producing female on Yamsky rookery was six years of age with a mean age of seven and was the only rookery where a younger age at first reproduction corresponded with lower concentrations of OCs. Females at Brat Chirpoyev did not appear to be negatively affected by OCs; however, half of those females successfully had pups despite having high mean concentrations of ∑PCB and ∑PBDE. These results suggest that OCs are not at high enough concentrations to affect reproduction or that SSL at Brat Chirpoyev have a higher threshold before experiencing negative biological effects in the form of reduced reproductive success.

This research is an initial attempt to understand the relationship between OCs and SSL reproductive parameters using longitudinal data. The concentrations of OCs in SSL pups are at levels suggested to impair normal physiological functions and therefore have the potential to negatively affect the population. Although our data suggest no consistent negative effects at these concentrations, OCs should not be ruled out as a contributing source to the SSL decline or failure to recover without more comprehensive OC analyses from branded SSL. Additional research should focus on obtaining paired mother-pup blood samples as well as multiple blood samples from the same animals over time. Ideally, this would occur concomitantly with continued hot-branding and resighting to better understand the relationship between the transference of contaminants and its effects on successive offspring for this species.

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Tables

Table 2.1. Reproductive success at four natal rookeries for female Steller sea lions branded in 2002, for which contaminants were quantified from Myers et al. 2008.

Natal Rookery branded, 2002	Sample size at each rookery	Females resighted with pup	Females resighted without pup	Females not resighted
Iony Is.	12	1	3	8
Kozlova Cape	17	9	0	8
Medny Is.	20	5	4	11
Yamsky	13	2	1	10
Total	**62**	**17**	**8**	**37**

Table 2.2. Reproductive success at nine natal rookeries for female Steller sea lions branded from 2001-2007.

Natal Rookery branded, 2001 - 2007	Sample size at each rookery	Females resighted with pup	Females resighted without pup
Medny Island	4	4	0
Kozlova Cape	3	3	0
Antsiferov	24	13	11
Lovushki	14	11	3
Brat Chirpoev	16	11	5
Srednego	8	1	7
Raykoke	12	7	5
Iony Island	12	4	8
Yamsky	10	5	5
Total	**103**	**59**	**44**

Table 2.3a. Generalized linear mixed effects models and AICc for reproductive success for female SSL (n = 25). Notation is such that, PBi = binomial value indicating successful (1) or unsuccessful (0) pup birth, NR = natal rookery (Iony Island, Kozlova Cape, Medny Island, Yamsky), Wt = female pup birth weight, PCB = ∑PCB (ww ng g^{-1}), DDT = ∑DDT (ww ng g^{-1}), and (1 | Tag) is the random effect, which are the alpha-numeric hot-brands applied to each female pup.

	Generalized linear mixed effects model	AICc
1	PBi = β_0 + (1 | Tag)	171.01
2	PBi = β_0 + β_1PCB + (1 | Tag)	172.82
3	PBi = β_0 + β_1Wt + (1 | Tag)	172.95
4	PBi = β_0 + β_1DDT + (1 | Tag)	172.97
5	PBi = β_0 + β_1NR + (1 | Tag)	174.31
6	PBi = β_0 + β_1NR + β_2Wt (1 | Tag)	174.74
7	PBi = β_0 + β_1PCB + β_2Wt + (1 | Tag)	174.90
8	PBi = β_0 + β_1DDT + β_2Wt + (1 | Tag)	175.02
9	PBi = β_0 + β_1PCB + β_2NR + β_3Wt + (1 | Tag)	175.93
10	PBi = β_0 + β_1DDT + β_2NR + β_3Wt + (1 | Tag)	176.33
11	PBi = β_0 + β_1DDT + β_2NR + (1 | Tag)	176.46
12	PBi = β_0 + β_1PCB + β_2NR + (1 | Tag)	176.47

Table 2.3b. Parameter estimations from the generalized linear mixed effects models for reproductive success (Table 2.3a), which includes the coefficients, standard errors, and p-values for each parameter; Wt = female pup birth weight, PCB = ∑PCB (ww ng g^{-1}), and DDT = ∑DDT (ww ng g^{-1}).

Model	Predictor	Coefficients	SE	p-value
5	Iony	-2.30	0.97	*0.01
	Kozlova	-0.65	0.52	0.13
	Medny	-1.22	0.55	0.33
	Yamsky	-1.97	1.01	0.81
6	Wt	-0.13	0.10	0.18
	Iony	0.82	2.43	0.74
	Kozlova	3.36	3.02	*0.05
	Medny	2.57	2.85	0.16
	Yamsky	2.19	3.22	0.39
9	PCB	-0.10	0.10	0.31
	Wt	-0.21	0.13	0.10
	Iony	3.09	3.28	0.35
	Kozlova	6.47	4.36	*0.04
	Medny	5.19	3.87	0.11
	Yamsky	5.05	4.32	0.26
10	DDT	-0.10	0.12	0.43
	Wt	-0.19	0.12	0.13
	Iony	2.44	3.90	0.44
	Kozlova	5.43	4.10	*0.04
	Medny	4.40	3.70	0.12
	Yamsky	4.16	4.12	0.31

Table 2.4a. Linear regression models and AICc for age at first reproduction for female SSL (n = 25). Notation is such that, Age = age at first observable reproduction, NR = natal rookery (Iony Island, Kozlova Cape, Medny Island, Yamsky), Wt = female pup birth weight, PCB = ∑PCB (ww ng g^{-1}), and DDT = ∑DDT (ww ng g^{-1}).

	Linear regression model	AICc
1	Age = $\beta_0 + \beta_1$NR	92.97
2	Age = $\beta_0 + \beta_1$DDT + β_2NR	95.03
3	Age = $\beta_0 + \beta_1$NR + β_2Wt	95.05
4	Age = $\beta_0 + \beta_1$PCB + β_2NR	95.39
5	Age = $\beta_0 + \beta_1$DDT + β_2NR + β_3DDT*NR	102.64
6	Age = $\beta_0 + \beta_1$NR + β_2Wt + β_3NR*Wt	102.95
7	Age = $\beta_0 + \beta_1$PCB + β_2NR + β_3PCB*NR	103.89
8	Age = β_0	105.12
9	Age = $\beta_0 + \beta_1$PCB + β_2Wt	105.84
10	Age = $\beta_0 + \beta_1$Wt	105.95
11	Age = $\beta_0 + \beta_1$DDT + β_2Wt	106.17
12	Age = $\beta_0 + \beta_1$PCB + β_2NR + β_3Wt + β_4NR*Wt	106.67
13	Age = $\beta_0 + \beta_1$PCB	106.74
14	Age = $\beta_0 + \beta_1$DDT	107.04
15	Age = $\beta_0 + \beta_1$DDT + β_2NR + β_3Wt + β_4NR*Wt	107.27
16	Age = $\beta_0 + \beta_1$PCB + β_2Wt + β_3PCB*Wt	108.84
17	Age = $\beta_0 + \beta_1$DDT + β_2Wt + β_3DDT*Wt	109.30

Table 2.4b. Parameter estimations from the linear regression models for age at first reproduction (Table 2.4a), which includes the coefficients, standard errors, and p-values for each parameter; Wt = female pup birth weight, PCB = ∑PCB, DDT = ∑DDT, natal rookeries, and the interaction between rookeries and Wt (eg: Iony*Wt).

Model	Predictor	Coefficients	SE	p-value
12	PCB	-0.11	0.09	0.27
	Iony	2.23	3.93	0.58
	Kozlova	10.35	5.17	0.18
	Medny	5.90	5.65	0.60
	Yamsky	-1.52	7.80	0.67
	Iony*Wt	0.29	0.16	0.09
	Kozlova*Wt	-0.44	0.21	*0.05
	Medny*Wt	-0.30	0.25	0.25
	Yamsky*Wt	-0.06	0.29	0.83
13	Intercept	5.97	0.51	*3.27e-11
	PCB	-0.08	0.08	0.35
14	Intercept	5.92	0.51	*4.6e-11
	DDT	-0.09	0.11	0.44
15	DDT	-0.11	0.11	0.35
	Iony	2.21	4.03	0.59
	Kozlova	8.78	4.59	0.24
	Medny	5.28	5.76	0.67
	Yamsky	-1.00	8.11	0.72
	Iony*Wt	0.28	0.16	0.10
	Kozlova*Wt	-0.40	0.20	0.06
	Medny*Wt	-0.28	0.26	0.30
	Yamsky*Wt	-0.08	0.30	0.78

Table 2.5a. Generalized linear mixed effects models and AICc for reproductive success for female SSL (n = 103). Notation is such that, PBi = binomial value indicating successful (1) or unsuccessful (0) pup birth, NR = natal rookery (Antsiferov, Brat Chirpoyev, Iony Island, Kozlova Cape, Lovushki, Medny Island, Raykoke, Srednego, and Yamsky), PCB = ∑PCB (lw mg g^{-1}), DDT = ∑DDT (lw mg g^{-1}), PBDE = ∑PBDE (lw mg g^{-1}), and (1 | Tag) is the random effect, which are the alpha-numeric hot-brands applied to each female pup.

	Generalized linear mixed effects model	AICc
1	PBi = $\beta_0 + \beta_1$NR + (1 | Tag)	506.14
2	PBi = $\beta_0 + \beta_1$PBDE + β_2NR + (1 | Tag)	507.84
3	PBi = $\beta_0 + \beta_1$PCB + β_2NR + (1 | Tag)	507.66
4	PBi = $\beta_0 + \beta_1$DDT + β_2NR + (1 | Tag)	508.24
5	PBi = β_0 + (1 | Tag)	512.30
6	PBi = $\beta_0 + \beta_1$DDT + β_2NR + β_3DDT*NR + (1 | Tag)	512.92
7	PBi = $\beta_0 + \beta_1$DDT + (1 | Tag)	513.33
8	PBi = $\beta_0 + \beta_1$PCB + β_2NR + β_3PCB*NR + (1 | Tag)	513.56
9	PBi = $\beta_0 + \beta_1$PCB + (1 | Tag)	514.27
10	PBi = $\beta_0 + \beta_1$PBDE + (1 | Tag)	514.31
11	PBi = $\beta_0 + \beta_1$PBDE + β_2NR + β_3PBDE*NR (1 | Tag)	519.61

Table 2.5b. Parameter estimations from the generalized linear mixed effects models for reproductive success (Table 2.5b), which includes the coefficients, standard errors, and p-values for each parameter; PCB = ∑PCB (lw mg g^{-1}), DDT = ∑DDT (lw mg g^{-1}), and PBDE = (lw mg g^{-1}).

Model	Predictor	Coefficients	SE	p-value
1	Antsiferov	-0.87	0.36	*0.02
	Brat Chirpoyev	-0.85	0.57	0.98
	Iony	-2.26	0.64	*0.03
	Kozlova	-1.28	1.03	0.69
	Lovushki	-0.82	0.57	0.94
	Medny	0.41	0.94	0.17
	Raykoke	-0.33	0.60	0.37
	Srednego	-4.06	1.38	*0.02
	Yamsky	-1.84	0.67	0.14
2	PBDE	-0.57	0.89	0.52
	Antsiferov	-0.64	0.50	0.20
	Brat Chirpoyev	-0.64	0.57	1.00
	Iony	-2.07	0.64	*0.03
	Kozlova	-1.06	1.03	0.68
	Lovushki	-0.62	0.57	0.98
	Medny	0.74	0.95	0.15
	Raykoke	-0.09	0.60	0.36
	Srednego	-3.85	1.38	*0.02
	Yamsky	-1.63	0.67	0.14
3	PCB	-0.05	0.44	0.13
	Antsiferov	-0.67	0.07	0.44
	Brat Chirpoyev	-0.63	0.57	0.95
	Iony	-2.12	0.64	*0.02
	Kozlova	-1.02	1.03	0.73
	Lovushki	-0.64	0.57	0.96
	Medny	0.72	0.95	0.14
	Raykoke	-0.07	0.61	0.32
	Srednego	-3.80	1.38	*0.02
	Yamsky	-1.68	0.67	0.13

Table 2.5b continued.

Model	Predictor	Coefficients	SE	p-value
4	DDT	-1.12e-3	0.09	0.99
	Antsiferov	-0.86	0.42	*0.04
	Brat Chirpoyev	-0.85	0.57	0.98
	Iony	-2.26	0.64	*0.03
	Kozlova	-1.28	1.04	0.69
	Lovushki	-0.82	0.57	0.94
	Medny	0.42	0.95	0.18
	Raykoke	-0.32	0.61	0.37
	Srednego	-4.06	1.38	*0.02
	Yamsky	-1.84	0.67	0.15

Table 2.6a. Linear regression models and AICc for age at first reproduction for female SSL (n = 103). Notation is such that, Age = age at first observable reproduction, NR = natal rookery (Antsiferov, Brat Chirpoyev, Iony Island, Kozlova Cape, Lovushki, Medny Island, Raykoke, Srednego, and Yamsky), PCB = ∑PCB (lw mg g^{-1}), DDT = ∑DDT (lw mg g^{-1}), and PBDE = ∑PBDE (lw mg g^{-1}).

	Linear regression model	AICc
1	Age = $\beta_0 + \beta_1$PCB + β_2NR + β_3PCB*NR	61.69
2	Age = $\beta_0 + \beta_1$PBDE + β_2NR + β_3PBDE*NR	64.92
3	Age = $\beta_0 + \beta_1$PBDE + β_2NR	67.63
4	Age = $\beta_0 + \beta_1$NR	69.34
5	Age = $\beta_0 + \beta_1$PCB + β_2NR	70.24
6	Age = $\beta_0 + \beta_1$DDT + β_2NR	72.84
7	Age = $\beta_0 + \beta_1$DDT + β_2NR + β_3DDT*NR	82.35
8	Age = $\beta_0 + \beta_1$DDT	90.85
9	Age = $\beta_0 + \beta_1$PBDE	92.16
10	Age = $\beta_0 + \beta_1$PCB	93.62
11	Age = β_0	94.23

Table 2.6b. Parameter estimations from the linear regression models for age at first reproduction (Table 2.6a), which includes the coefficients, standard errors, and p-values for each parameter; PCB = ∑PCB (lw mg g^{-1}), DDT = ∑DDT (lw mg g^{-1}), PBDE = ∑PBDE (lw mg g^{-1}), natal rookeries, and the interaction between rookeries and OCs (eg: Antsiferov*PCB).

Model	Predictor	Coefficients	SE	p-value
1	PCB	0.04	0.02	0.07
	Antsiferov	2.09	0.11	*2.00e-16
	Brat Chirpoyev	2.59	0.18	*5.80e-3
	Iony	2.94	0.18	*8.09e-06
	Kozlova	2.29	0.49	0.69
	Lovushki	2.08	0.18	0.98
	Medny	2.28	0.36	0.59
	Raykoke	2.10	0.23	0.97
	Srednego	3.20	0.27	*6.84e-05
	Yamsky	2.82	0.18	*7.28e-05
	Antsiferov*PCB	-0.02	0.02	0.23
	Brat Chirpoyev*PCB	-0.11	0.03	*1.96e-3
	Iony*PCB	-0.09	0.04	*0.05
	Kozlova*PCB	-0.02	0.09	0.81
	Lovushki*PCB	8.33e-4	0.04	0.98
	Medny*PCB	-0.07	0.06	0.24
	Raykoke*PCB	2.89e-3	0.04	0.95
	Srednego*PCB	-0.15	0.05	*1.71e-3
	Yamsky*PCB	-0.07	0.04	0.06

Table 2.6b continued.

Model	Predictor	Coefficients	SE	p-value
2	PBDE	0.12	0.28	0.65
	Antsiferov	2.20	0.12	* 2e-16
	Brat Chirpoyev	2.61	0.20	*0.04
	Iony	3.05	0.20	*6.95e-05
	Kozlova	2.25	0.43	0.90
	Lovushki	2.08	0.20	0.56
	Medny	2.22	0.61	0.96
	Raykoke	2.06	0.23	0.56
	Srednego	3.38	0.26	*1.41e-05
	Yamsky	2.93	0.22	*1.47e-3
	Antsiferov*PBDE	-0.49	0.21	*0.02
	Brat Chirpoyev*PBDE	-0.96	0.44	*0.03
	Iony*PBDE	-0.86	0.48	0.08
	Kozlova*PBDE	0.21	1.03	0.83
	Lovushki*PBDE	0.34	0.45	0.45
	Medny*PBDE	-0.32	1.08	0.77
	Raykoke*PBDE	0.50	0.51	0.33
	Srednego*PBDE	-1.86	0.51	*6.89e-4
	Yamsky*PBDE	-0.72	0.51	0.17
3	PBDE	-0.28	0.15	0.07
	Antsiferov	2.36	0.09	* 2e-16
	Brat Chirpoyev	2.38	0.10	0.81
	Iony	2.89	0.11	*7.52e-06
	Kozlova	2.48	0.19	0.51
	Lovushki	2.35	0.11	0.97
	Medny	2.27	0.17	0.63
	Raykoke	2.43	0.11	0.50
	Srednego	2.73	0.13	*4.63e-3
	Yamsky	2.81	0.12	*2.2e-4

Table 2.6b continued.

Model	Predictor	Coefficients	SE	p-value
4	Antsiferov	2.25	0.07	*2e-16
	Brat Chirpoyev	2.27	0.10	0.83
	Iony	2.79	0.11	*6.02e-06
	Kozlova	2.38	0.20	0.50
	Lovushki	2.25	0.11	0.96
	Medny	2.12	0.17	0.46
	Raykoke	2.32	0.11	0.53
	Srednego	2.61	0.13	*6.69e-3
	Yamsky	2.71	0.12	*2.11e-4
5	PCB	-0.01	0.01	0.32
	Antsiferov	2.29	0.08	* 2e-16
	Brat Chirpoyev	2.33	0.10	0.75
	Iony	2.83	0.11	*9.47e-06
	Kozlova	2.43	0.20	0.46
	Lovushki	2.30	0.11	0.94
	Medny	2.19	0.18	0.55
	Raykoke	2.38	0.11	0.45
	Srednego	2.68	0.13	*4.75e-3
	Yamsky	2.75	0.12	*2.44e-4
6	DDT	-0.08	0.07	0.26
	Antsiferov	2.34	0.11	*2e-16
	Brat Chirpoyev	2.35	0.10	0.90
	Iony	2.86	0.12	*1.68e-5*
	Kozlova	2.46	0.20	0.55
	Lovushki	2.34	0.11	1.00
	Medny	2.24	0.17	0.56
	Raykoke	2.42	0.11	0.46
	Srednego	2.69	0.13	*8.51e-3
	Yamsky	2.79	0.12	*4.34e-4

Table 2.6b continued.

Model	Predictor	Coefficients	SE	p-value
7	DDT	0.04	0.14	0.76
	Antsiferov	2.20	0.17	* 2e-16
	Brat Chirpoyev	2.58	0.27	0.15
	Iony	3.14	0.26	*4.15e-4
	Kozlova	2.88	1.17	0.56
	Lovushki	1.98	0.28	0.44
	Medny	2.47	0.77	0.72
	Raykoke	2.04	0.35	0.66
	Srednego	2.71	0.34	0.13
	Yamsky	3.00	0.28	*5.73e-3
	Antsiferov*DDT	-0.21	0.09	*0.02
	Brat Chirpoyev*DDT	-0.34	0.22	0.13
	Iony*DDT	-0.43	0.23	0.07
	Kozlova*DDT	-0.55	1.17	0.64
	Lovushki*DDT	0.20	0.23	0.38
	Medny*DDT	-0.28	0.51	0.59
	Raykoke*DDT	0.17	0.26	0.52
	Srednego*DDT	-0.14	0.29	0.63
	Yamsky*DDT	-0.36	0.26	0.17
8	Intercept	2.60	0.09	*2e-16
	DDT	-0.18	0.08	*0.02
9	Intercept	2.53	0.08	*2e-16
	PBDE	-0.34	0.17	*0.05
10	Intercept	2.48	0.07	*2e-16
	PCB	-0.02	0.01	0.12
11	Intercept	2.39	0.04	*2e-16

Table 2.7a. Linear regression models and AICc for age at first reproduction, limited to female SSL with pups (n = 59). Notation is such that, Age = age at first observable reproduction, NR = natal rookery (Antsiferov, Brat Chirpoyev, Iony Island, Kozlova Cape, Lovushki, Medny Island, Raykoke, Srednego, and Yamsky), PCB = ∑PCB (lw mg g^{-1}), DDT = ∑DDT (lw mg g^{-1}), and PBDE = ∑PBDE (lw mg g^{-1}).

	Linear regression model	AICc
1	Age = $\beta_0 + \beta_1$PCB + β_2NR	-14.34
2	Age = $\beta_0 + \beta_1$NR	-13.77
3	Age = $\beta_0 + \beta_1$DDT + β_2NR	-12.07
4	Age = $\beta_0 + \beta_1$PBDE + β_2NR	-10.78
5	Age = $\beta_0 + \beta_1$PCB	-8.08
6	Age = $\beta_0 + \beta_1$DDT	-5.54
7	Age = β_0	-5.21
8	Age = $\beta_0 + \beta_1$PBDE	-3.28
9	Age = $\beta_0 + \beta_1$PCB + β_2NR + β_3PCB*NR	1.89
10	Age = $\beta_0 + \beta_1$PBDE + β_2NR + β_3PBDE*NR	2.04
11	Age = $\beta_0 + \beta_1$DDT + β_2NR + β_3DDT*NR	4.38

Table 2.7b. Parameter estimations from the linear regression models for age at first reproduction, limited to female SSL with pups (Table 2.7a), which includes the coefficients, standard errors, and p-values for each parameter; PCB = ∑PCB (lw mg g^{-1}), DDT = ∑DDT (lw mg g^{-1}), PBDE = ∑PBDE (lw mg g^{-1}), natal rookeries, and the interaction between rookeries and OCs (eg: Antsiferov*PCB).

Model	Predictor	Coefficients	SE	p-value
1	PCB	-0.06	0.04	0.09
	Antsiferov	2.37	0.08	*2e-16
	Brat Chirpoyev	2.34	0.08	0.66
	Iony	2.53	0.11	0.14
	Kozlova	2.52	0.12	0.23
	Lovushki	2.28	0.08	0.23
	Medny	2.27	0.11	0.33
	Raykoke	2.33	0.09	0.64
	Srednego	2.61	0.20	0.23
	Yamsky	2.73	0.10	*5.75e-4
2	Antsiferov	2.26	0.05	*2e-16
	Brat Chirpoyev	2.21	0.08	0.49
	Iony	2.44	0.11	0.11
	Kozlova	2.38	0.12	0.34
	Lovushki	2.17	0.08	0.24
	Medny	2.12	0.11	0.19
	Raykoke	2.20	0.09	0.46
	Srednego	2.45	0.20	0.35
	Yamsky	2.64	0.10	*4.46e-4

Table 2.7b continued.

Model	Predictor	Coefficients	SE	p-value
3	DDT	-0.04	0.04	0.30
	Antsiferov	2.31	0.07	*2.00e-16
	Brat Chirpoyev	2.26	0.08	0.52
	Iony	2.47	0.11	0.14
	Kozlova	2.42	0.12	0.38
	Lovushki	2.21	0.08	0.24
	Medny	2.19	0.11	0.29
	Raykoke	2.26	0.09	0.57
	Srednego	2.53	0.20	0.27
	Yamsky	2.68	0.10	*5.07e-4
4	PBDE	-0.02	0.13	0.85
	Antsiferov	2.27	0.07	*2.00e-16
	Brat Chirpoyev	2.22	0.08	0.49
	Iony	2.44	0.11	0.12
	Kozlova	2.39	0.12	0.35
	Lovushki	2.18	0.08	0.24
	Medny	2.13	0.11	0.22
	Raykoke	2.21	0.09	0.46
	Srednego	2.46	0.20	0.35
	Yamsky	2.65	0.10	*5.11e-4

Table 2.7b continued.

Model	Predictor	Coefficients	SE	p-value
9	PCB	-0.017	0.08	0.83
	Antsiferov	2.29	0.15	*2.00e-16
	Brat Chirpoyev	2.47	0.22	0.43
	Iony	4.68	1.05	*0.03
	Kozlova	2.20	0.59	0.89
	Lovushki	2.20	0.20	0.66
	Medny	2.38	0.36	0.82
	Raykoke	2.17	0.30	0.69
	Srednego	2.49	0.20	0.33
	Yamsky	2.85	0.23	*0.02
	Antsiferov*PCB	-0.07	0.05	0.16
	Brat Chirpoyev*PCB	-0.11	0.11	0.31
	Iony*PCB	-1.52	0.71	*0.04
	Kozlova*PCB	0.10	0.27	0.73
	Lovushki*PCB	1.55e-3	0.11	0.99
	Medny*PCB	-0.09	0.16	0.55
	Raykoke*PCB	0.03	0.15	0.84
	Srednego*PCB	NA	NA	NA
	Yamsky*PCB	-0.13	0.13	0.35

Table 2.7b continued.

Model	Predictor	Coefficients	SE	p-value
10	PBDE	0.14	0.24	0.57
	Antsiferov	2.21	0.11	*2.00e-16
	Brat Chirpoyev	2.27	0.16	0.69
	Iony	2.93	0.25	*6.68e-3
	Kozlova	2.25	0.29	0.89
	Lovushki	2.12	0.15	0.57
	Medny	2.23	0.40	0.96
	Raykoke	1.98	0.24	0.33
	Srednego	2.39	0.19	0.34
	Yamsky	2.88	0.22	*3.63e-3
	Antsiferov*PBDE	-0.01	0.18	0.96
	Brat Chirpoyev*PBDE	-0.30	0.37	0.42
	Iony*PBDE	-1.85	0.77	*0.02
	Kozlova*PBDE	0.20	0.68	0.77
	Lovushki*PBDE	3.51e-3	0.35	0.99
	Medny*PBDE	-0.33	0.71	0.64
	Raykoke*PBDE	0.43	0.57	0.45
	Srednego*PBDE	NA	NA	NA
	Yamsky*PBDE	-0.75	0.50	0.14

Table 2.8a. Linear regression models and AICc for age at first reproduction, limited to female SSL with no pups (n = 44). Notation is such that, Age = age at first observable reproduction, NR = natal rookery (Antsiferov, Brat Chirpoyev, Iony Island, Lovushki, Raykoke, Srednego, and Yamsky), PCB = ∑PCB (lw mg g^{-1}), DDT = ∑DDT (lw mg g^{-1}), and PBDE = ∑PBDE (lw mg g^{-1}).

	Linear regression model	AICc
1	Age = $\beta_0 + \beta_1$PCB + β_2NR + β_3PCB*NR	192.88
2	Age = $\beta_0 + \beta_1$PBDE + β_2NR + β_3PBDE*NR	196.63
3	Age = $\beta_0 + \beta_1$PBDE + β_2NR	197.90
4	Age = $\beta_0 + \beta_1$PBDE	199.99
5	Age = $\beta_0 + \beta_1$DDT	201.82
6	Age = $\beta_0 + \beta_1$PCB	203.09
7	Age = $\beta_0 + \beta_1$NR	203.22
8	Age = $\beta_0 + \beta_1$PCB + β_2NR	203.84
9	Age = β_0	204.12
10	Age = $\beta_0 + \beta_1$DDT + β_2NR	205.15
11	Age = $\beta_0 + \beta_1$DDT + β_2NR + β_3DDT*NR	215.32

Table 2.8b. Parameter estimations from the linear regression models for age at first reproduction, limited to female SSL with no pups (Table 2.8a), which includes the coefficients, standard errors, and p-values for each parameter; PCB = ∑PCB (lw mg g^{-1}), DDT = ∑DDT (lw mg g^{-1}), PBDE = ∑PBDE (lw mg g^{-1}), natal rookeries, and the interaction between rookeries and OCs (eg: Antsiferov*PCB).

Model	Predictor	Coefficients	SE	p-value
1	PCB	0.46	0.17	*1.01e-2
	Antsiferov	3.00	0.89	*2.06e-3
	Brat Chirpoyev	10.30	1.65	*1.15e-4
	Iony	10.52	1.26	*1.63e-06
	Kozlova	NA	NA	NA
	Lovushki	-0.11	3.58	0.39
	Medny	NA	NA	NA
	Raykoke	4.52	2.06	0.47
	Srednego	10.27	1.54	*5.24e-05
	Yamsky	8.74	1.37	*2.32e-4
	Antsiferov*PCB	-0.02	0.48	0.96
	Brat Chirpoyev*PCB	-1.23	0.27	*9.32e-05
	Iony*PCB	-0.94	0.27	*1.441e-3
	Kozlova*PCB	NA	NA	NA
	Lovushki*PCB	0.68	0.59	0.26
	Medny*PCB	NA	NA	NA
	Raykoke*PCB	-0.13	0.35	0.72
	Srednego*PCB	-1.03	0.26	*4.95e-4
	Yamsky*PCB	-0.69	0.25	*0.01

Table 2.8b continued.

Model	Predictor	Coefficients	SE	p-value
2	PBDE	0.53	2.25	0.82
	Antsiferov	4.88	1.03	*4.66e-05
	Brat Chirpoyev	10.70	1.86	*3.81e-3
	Iony	11.02	1.49	*2.69e-4
	Kozlova	NA	NA	NA
	Lovushki	4.06	2.28	0.72
	Medny	NA	NA	NA
	Raykoke	5.51	1.80	0.73
	Srednego	11.27	1.63	*4.87e-4
	Yamsky	8.90	1.68	*0.02
	Antsiferov*PBDE	-4.58	6.07	0.46
	Brat Chirpoyev*PBDE	-10.88	3.77	*0.01
	Iony*PBDE	-6.05	3.26	0.07
	Kozlova	NA	NA	NA
	Lovushki*PBDE	5.73	4.90	0.25
	Medny*PBDE	NA	NA	NA
	Raykoke*PBDE	1.42	3.61	0.70
	Srednego*PBDE	-9.70	3.35	*0.01
	Yamsky*PBDE	-3.59	3.86	0.36
3	PBDE	-3.49	1.26	*8.74e-3
	Antsiferov	6.50	0.77	*5.53e-10
	Brat Chirpoyev	7.59	1.05	0.31
	Iony	10.24	0.90	*2.01e-4
	Kozlova	NA	NA	NA
	Lovushki	8.12	1.27	0.21
	Medny	NA	NA	NA
	Raykoke	7.99	1.05	0.16
	Srednego	8.71	0.94	*0.02
	Yamsky	9.06	1.05	*0.02

Table 2.8b continued.

Model	Predictor	Coefficients	SE	p-value
8	PCB	-0.16	0.11	0.15
	Antsiferov	5.84	0.81	*1.66e-08
	Brat Chirpoyev	6.92	1.13	0.34
	Iony	9.44	0.98	*7.30e-4
	Kozlova	NA	NA	NA
	Lovushki	7.65	1.37	0.19
	Medny	NA	NA	NA
	Raykoke	7.33	1.13	0.19
	Srednego	8.05	1.01	*0.04
	Yamsky	8.49	1.12	*0.02
10	DDT	-1.05	1.03	0.31
	Antsiferov	6.37	1.41	*6.41e-05
	Brat Chirpoyev	6.99	1.17	0.60
	Iony	9.83	1.03	*1.94e-3
	Kozlova	NA	NA	NA
	Lovushki	7.88	1.38	0.28
	Medny	NA	NA	NA
	Raykoke	7.70	1.14	0.25
	Srednego	8.14	1.06	0.10
	Yamsky	8.71	1.20	0.06

Table 2.8b continued.

Model	Predictor	Coefficients	SE	p-value
11	DDT	0.91	2.32	0.70
	Antsiferov	3.98	2.88	0.18
	Brat Chirpoyev	16.12	5.30	*0.03
	Iony	11.89	3.35	*0.03
	Kozlova	NA	NA	NA
	Lovushki	1.55	4.70	0.61
	Medny	NA	NA	NA
	Raykoke	4.45	4.80	0.92
	Srednego	7.78	3.80	0.33
	Yamsky	9.29	3.96	0.19
	Antsiferov*DDT	-3.88	1.93	*0.05
	Brat Chirpoyev*DDT	-11.65	5.18	*0.03
	Iony*DDT	-4.25	2.89	0.15
	Kozlova*DDT	NA	NA	NA
	Lovushki*DDT	3.53	3.84	0.37
	Medny*DDT	NA	NA	NA
	Raykoke*DDT	0.67	3.81	0.86
	Srednego*DDT	-1.58	3.40	0.64
	Yamsky*DDT	-2.64	3.78	0.49

Figures

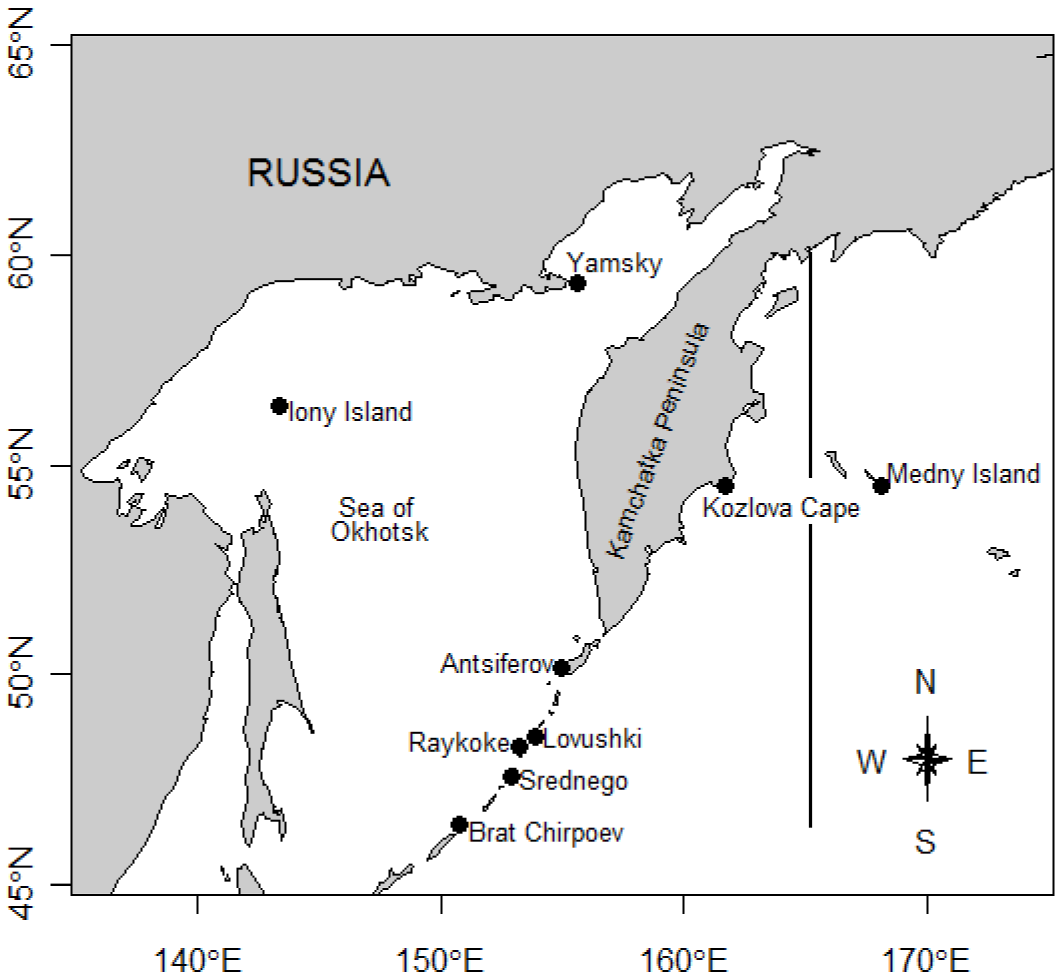

Figure 2.1. Map of Steller sea lion rookeries where hot-branding occurred. The black line at 165^0 E approximates the delineation between the Asian and western populations from Phillips et al. 2011.

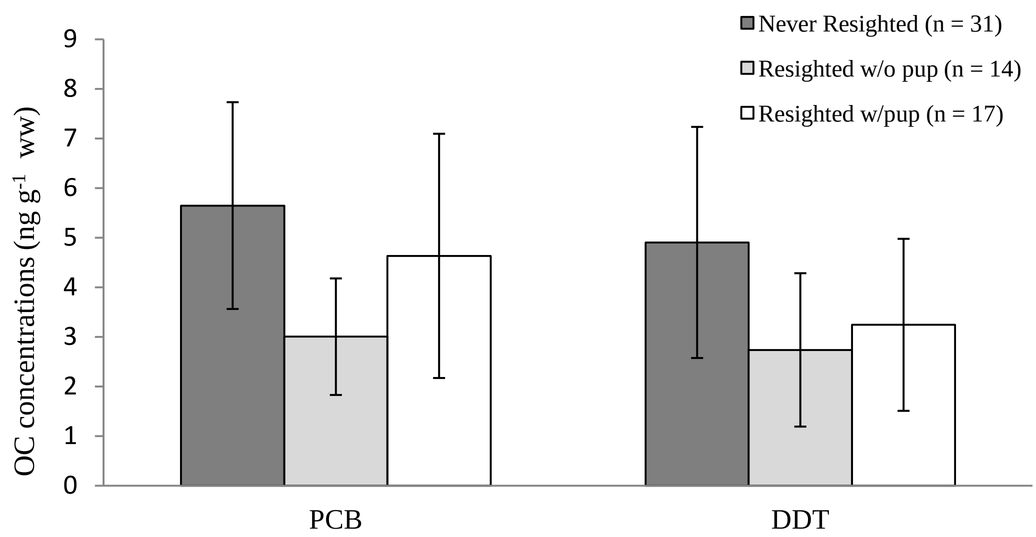

Figure 2.2. Mean, with 95% confidence intervals, ∑PCB and ∑DDT concentrations (ng g^{-1} ww) from whole blood for female SSL hot-branded in 2002 that were never resighted and those observed without and with pups.

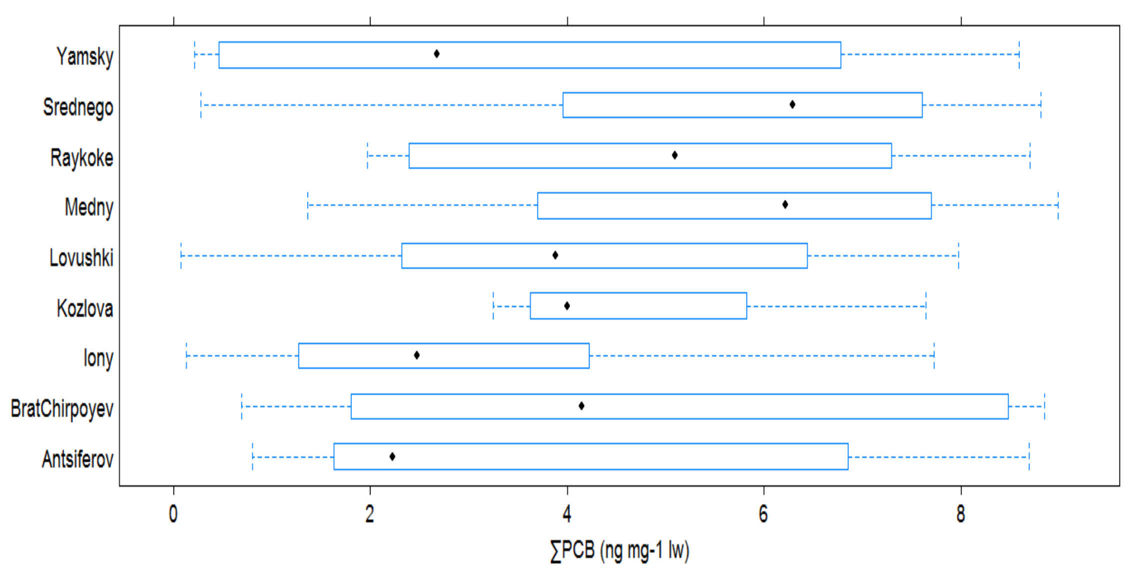

Figure 2.3. Range of ∑PCB concentrations (ng mg^{-1} lw) from blood serum at each natal rookery. The mean (black circle) is shown in the box bounded by the 25th and 75th percentiles and dashed lines show minimum and maximum ∑PCB concentrations.

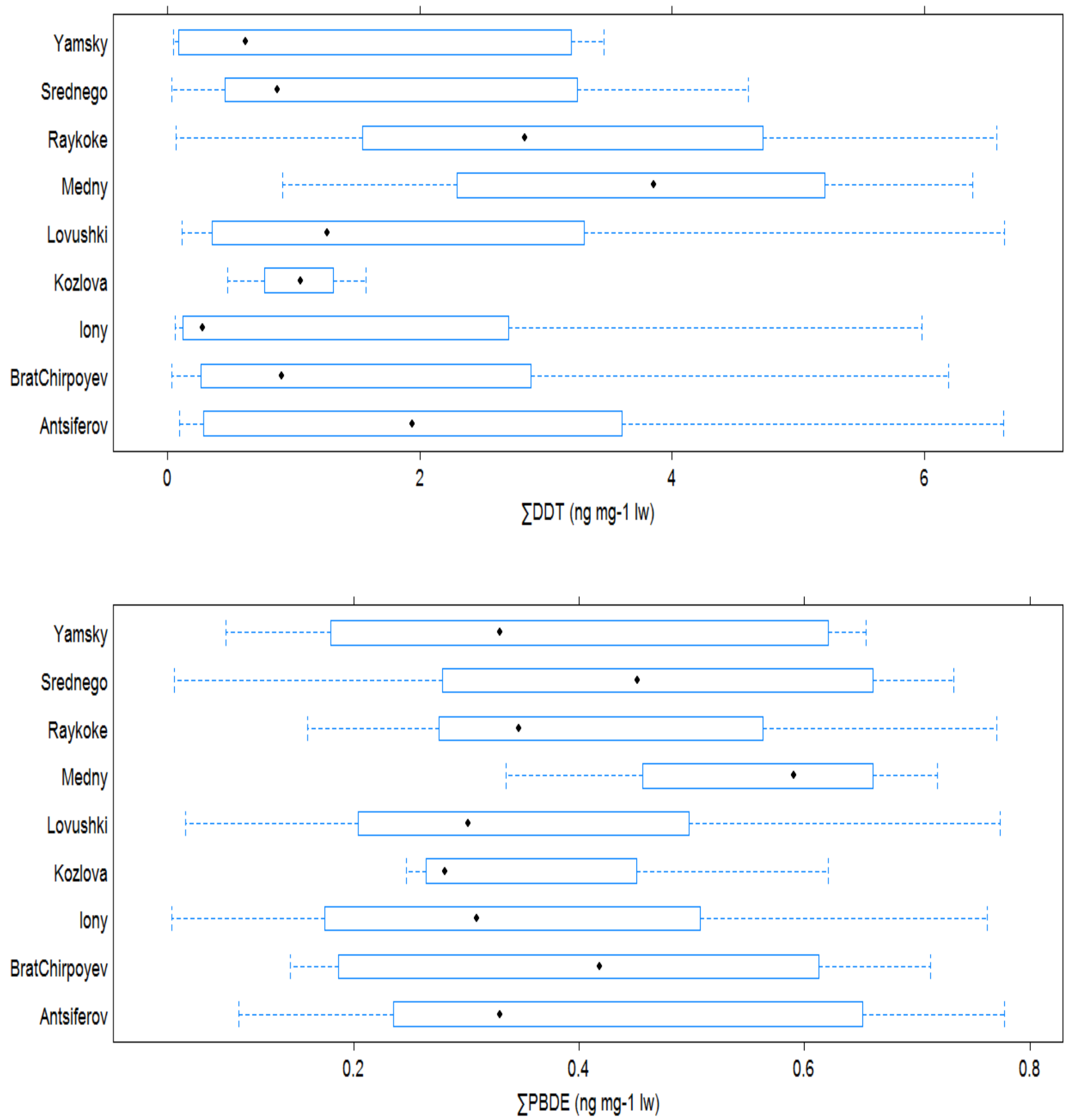

Figure 2.4. Range of ∑DDT and ∑PBDE concentrations (ng mg^{-1} lw) from blood serum, respectively, at each natal rookery. The mean (black circle) is shown in the box bounded by the 25th and 75th percentiles and dashed lines show minimum and maximum ∑PBDE concentrations.

Appendices

Appendix 2.1. Parameter estimations from Table 2.3a mixed effects models of reproductive success for female SSL (n = 25).

Model	Predictor	Coefficients	SE	p-value
1	(1|Tag)	-1.24	0.34	*2.48e-4
2	Intercept	-1.41	0.46	*2.44e-3
	PCB	0.04	0.07	0.60
3	Intercept	-0.31	2.40	0.90
	Wt	-0.03	0.08	0.70
4	Intercept	-1.35	0.47	*3.73e-3
	DDT	0.04	0.10	0.72
7	Intercept	-0.88	2.78	0.75
	PCB	0.03	0.08	0.68
	Wt	-0.02	0.09	0.85
8	Intercept	-0.63	2.86	0.82
	DDT	0.02	0.11	0.84
	Wt	-0.02	0.09	0.80
11	DDT	0.01	0.10	0.88
	Iony	-2.34	1.02	*0.02
	Kozlova	-0.70	0.65	0.28
	Medny	-1.26	0.61	*0.04
	Yamsky	-2.00	1.03	*0.05
12	PCB	6.84e-3	0.08	0.93
	Iony	-2.32	1.01	*0.02
	Kozlova	-0.69	0.70	0.33
	Medny	-1.24	0.59	*0.04
	Yamsky	-1.99	1.03	*0.05

Appendix 2.2. Parameter estimations from Table 2.4a linear regression models of age at first reproduction for female SSL (n = 25).

Model	Predictor	Coefficients	SE	p-value
1	Iony	8.75	0.65	*8.85e-12
	Kozlova	5.00	0.43	*9.85e-05
	Medny	5.11	0.43	*1.38e-04
	Yamsky	5.00	0.75	*1.12e-03
2	DDT	-0.09	0.69	0.29
	Iony	9.02	0.08	*3.23e-1
	Kozlova	5.34	0.78	*1.39e-4
	Medny	5.33	0.78	*1.31e-4
	Yamsky	5.22	0.99	*1.04e-3
3	Iony	6.72	1.98	*2.91e-3
	Kozlova	2.45	0.92	*1.53e-4
	Medny	2.70	0.86	*1.38e-4
	Yamsky	2.39	1.13	*1.01e-3
	Wt	0.08	0.08	0.29
4	PCB	-0.06	0.06	0.37
	Iony	8.95	0.69	*3.09e-11
	Kozlova	5.37	0.81	*2.51e-4
	Medny	5.28	0.79	*1.46e-4
	Yamsky	5.18	1.00	*1.19e-3
5	DDT	-0.98	0.47	*0.05
	Iony	11.70	1.56	*8.75e-07
	Kozlova	5.24	1.66	*1.16e-3
	Medny	5.15	1.66	*1.06e-3
	Yamsky	5.90	1.95	*8.61e-3
	Iony*DDT	0.71	0.28	*0.02
	Kozlova*DDT	0.91	0.48	0.08
	Medny*DDT	0.96	0.50	0.07
	Yamsky*DDT	0.61	0.60	0.33

Appendix 2.2 continued.

Model	Predictor	Coefficients	SE	p-value
6	Iony	1.06	3.84	0.79
	Kozlova	5.62	4.99	0.37
	Medny	5.94	6.87	0.49
	Yamsky	-3.35	8.61	0.62
	Wt	0.32	0.16	0.06
	Iony*Wt	0.32	0.16	0.06
	Kozlova*Wt	-0.34	0.19	0.09
	Medny*Wt	-0.35	0.25	0.19
	Yamsky*Wt	-0.05	0.29	0.87
7	PCB	-0.88	0.47	0.08
	Iony	11.64	1.69	*2.55e-06
	Kozlova	5.27	1.79	*2.40e-3
	Medny	5.07	1.85	*2.43 e-3
	Yamsky	6.22	2.38	*0.04
	Iony*PCB	0.66	0.26	*0.02
	Kozlova*PCB	0.83	0.48	0.10
	Medny*PCB	0.89	0.52	0.11
	Yamsky*PCB	0.47	0.68	0.50
8	Intercept	5.64	0.34	*7.27e-14
9	Intercept	11.40	2.91	*7.45e-4
	PCB	-0.15	0.09	0.11
	Wt	-0.18	0.09	0.07
10	Intercept	9.03	2.64	*2.32e-3
	Wt	-0.12	0.09	0.21
11	Intercept	11.50	3.01	*9.23e-4
	DDT	-0.19	0.12	0.13
	Wt	-0.18	0.10	0.07
16	Intercept	10.16	4.62	*0.04
	PCB	0.22	1.04	0.83
	Wt	-0.13	0.17	0.44
	PCB*Wt	-0.01	0.04	0.73

Appendix 2.2 continued.

Model	Predictor	Coefficients	SE	p-value
17	Intercept	11.93	4.28	*0.01
	DDT	-0.33	0.99	0.74
	Wt	-0.20	0.15	0.19
	DDT*Wt	5.46e-3	0.04	0.89

Appendix 2.3. Parameter estimations from Table 2.5a mixed effects models of reproductive success of female SSL (n = 103). Model 6 estimations from Table 2.5a are omitted due to inestimable parameters.

Model	Predictor	Coefficients	SE	p-value
5	(1|Tag)	-1.26	0.19	6.73e-11
7	Intercept	-1.46	0.28	*1.41e-07
	DDT	0.10	0.10	0.31

Appendix 2.3 continued.

Model	Predictor	Coefficients	SE	p-value
8	PCB	-0.18	0.12	0.14
	Antsiferov	-0.13	0.55	0.82
	Brat Chirpoyev	-2.09	0.91	*0.03
	Iony	-1.60	0.99	0.14
	Kozlova	-1.25	2.52	0.66
	Lovushki	-0.20	0.88	0.93
	Medny	-1.68	2.15	0.47
	Raykoke	1.11	1.12	0.27
	Srednego	-5.24	3.70	0.18
	Yamsky	-1.43	0.91	0.15
	Antsiferov*PCB	-0.05	0.10	0.60
	Brat Chirpoyev*PCB	0.48	0.18	*9.04e-3
	Iony*PCB	-0.01	0.31	0.96
	Kozlova*PCB	0.18	0.48	0.70
	Lovushki* PCB	0.01	0.20	0.96
	Medny*PCB	0.53	0.35	0.13
	Raykoke*PCB	-0.10	0.22	0.64
	Srednego*PCB	0.42	0.56	0.45
	Yamsky*PCB	0.08	0.22	0.73
9	Intercept	-1.20	0.34	*3.67e-4
	PCB	-0.01	0.07	0.84
10	Intercept	-1.24	0.41	*2.25e-3
	PBDE	-0.04	0.97	0.96

Appendix 2.3 continued.

Model	Predictor	Coefficients	SE	p-value
11	PBDE	-1.13	1.69	0.50
	Antsiferov	-0.39	0.73	0.60
	Brat Chirpoyev	-1.64	1.14	0.27
	Iony	-2.00	1.24	0.19
	Kozlova	-1.27	2.40	0.71
	Lovushki	-0.57	1.09	0.87
	Medny	0.55	3.76	0.80
	Raykoke	1.83	1.37	0.11
	Srednego	-4.68	3.16	0.18
	Yamsky	-2.07	1.32	0.20
	Antsiferov*PBDE	0.05	1.16	0.97
	Brat Chirpoyev*PBDE	3.29	2.61	0.21
	Iony*PBDE	0.60	3.16	0.85
	Kozlova*PBDE	1.13	5.72	0.84
	Lovushki*PBDE	0.44	2.6035	0.87
	Medny*PBDE	0.87	6.5956	0.90
	Raykoke*PBDE	-4.20	3.24	0.20
	Srednego*PBDE	2.99	7.03	0.67
	Yamsky*PBDE	1.89	3.10	0.54

Appendix 2.4. Parameter estimations from Table 2.7a linear regression models of age at first reproduction for female SSL with pups (n = 59).

Model	Predictor	Coefficients	SE	p-value
5	Intercept	2.44	0.08	*2.00e-16
	PCB	-0.09	0.04	*0.03
6	Intercept	2.35	0.06	*2.00e-16
	DDT	-0.06	0.04	0.12
7	Intercept	2.27	0.03	*2.00e-16
8	Intercept	2.30	0.07	*2.00e-16
	PBDE	-0.08	0.15	0.60
11	DDT	0.01	0.06	0.85
	Antsiferov	2.25	0.09	*2e-16
	Brat Chirpoyev	2.29	0.14	0.78
	Iony	2.61	0.19	0.07
	Kozlova	2.72	0.49	0.34
	Lovushki	2.16	0.15	0.55
	Medny	2.37	0.34	0.72
	Raykoke	2.04	0.21	0.34
	Srednego	2.42	0.21	0.40
	Yamsky	2.90	0.18	*9.17e-4
	Antsiferov*DDT	-0.04	0.05	0.50
	Brat Chirpoyev*DDT	-0.08	0.10	0.45
	Iony*DDT	-0.21	0.17	0.23
	Kozlova*DDT	-0.36	0.48	0.45
	Lovushki*DDT	-3.21e-3	0.11	0.98
	Medny*DDT	-0.15	0.18	0.41
	Raykoke*DDT	0.08	0.13	0.52
	Srednego*DDT	NA	NA	NA
	Yamsky*DDT	-0.25	0.14	0.07

Appendix 2.5. Parameter estimations from Table 2.8a linear regression models of age at first reproduction for female SSL with no pups (n = 44).

Model	Predictor	Coefficients	SE	p-value
4	Intercept	8.30	0.68	*2.42e-15
	PBDE	-3.68	1.43	*0.01
5	Intercept	9.02	1.10	*2.92e-10
	DDT	-2.16	1.00	*0.04
6	Intercept	7.81	0.67	*8.6e-15
	PCB	-0.22	0.12	0.08
7	Antsiferov	5.09	0.64	*1.43e-09
	Brat Chirpoyev	6.00	1.14	0.43
	Iony	8.88	0.98	*4.49e-4
	Kozlova	NA	NA	NA
	Lovushki	6.67	1.38	0.26
	Medny	NA	NA	NA
	Raykoke	6.40	1.14	0.26
	Srednego	7.14	1.02	*0.05
	Yamsky	7.80	1.14	*0.02
9	Intercept	6.77	0.36	*2.00e-16

General Conclusions

Survival rates estimated in this study were consistent with previous studies showing lower survival in the first year and greater survival probability for adult SSL. Compared to other studies, age-specific estimates of survival within the first year (age 0) for SSL from the eastern population range from 0.523 – 0.762 and age eight estimates range from 0.808 – 0.941 (Hastings et al. 2011), and from the western population survival estimates within the first year range from 0.725 – 0.782 and age eight estimates range from 0.856 – 0.875 (Pendleton et al. 2006; York 1994). The survival estimate in the Russian Far East for SSL within their first year ranged from 0.657 – 0.670 and from 0.858 – 0.904 at nine years of age (Burkanov, unpublished data) and at Kozlova Cape, survival to age three was estimated to be 0.578, and 0.866 for age seven (Burdin et al. 2009). The probability of survival at Iony Island and Yamsky within the first year ranged from 0.38 (0.25, 0.52) – 0.41 (0.34, 0.48), which were lower than may be expected when compared to previous studies and are not likely attributable to OC concentration. These two rookeries were shown to first have pups at 6 – 7 years of age, whereas other rookeries where shown to first have pups before 6 years of age. Iony Island and Yamsky may be rookeries of concern, because if first year survival is low and females are later to produce offspring, these rookeries may be more at risk of being disproportionately affected by environmental perturbations or recovery from such disturbances. However, when resighting effort at these locations was considered, the estimates for survival and reproductive parameters may have been biased due to inconsistent and sparse resighting effort.

The probability of resighting a marked SSL depended on it being within the study region and visible. The resighting of SSL often occurs during the summer breeding season and increases with age (Hastings et al. 2011). The probability of resighting in our study ranged from 0.48 – 0.87 with a geometric mean of 0.67, which are comparable values to 0.54 – 0.79 found by Hastings et al. (2009) and the average resighting probability of 0.78 found by Hastings et al. (2011). The lower estimates of resighting probability in our study are likely due to multiple years of no resighting effort on Iony Island and Yamsky and could be lower than 0.48 if Antsiferov and Tuleny rookeries, which receive much more consistent resighting effort, had not been included in the same stratum. However, these values may not be low for segments of the declining western population; for example, the probability of resighting at the Marmot Island rookery was as low as 0.017 in the 1980's and fluctuated throughout much of the study with probabilities up to 0.836 in 2001 (Pendleton et al. 2006).

The haulout location on Bering Island is frequented by SSL from Medny Island and had the highest probability of resighting followed by Kozlova Cape and Medny Island. The proximity of Medny and Bering Islands may increase the resighting probability of SSL hauled out on Bering Island opposed to traveling west toward the Kamchatka Peninsula, which is approximately 200 miles further, resulting in more time spent in open water thereby reducing resighting opportunity.

The most contaminated rookery was Medny Island for females sampled from 2001 – 2007 and Kozlova Cape for females sampled from 2002, but reproductive success estimates were some of the highest for these locations. When OCs did appear to have a negative impact on reproductive success or age at first reproduction, the model fit was poor indicating that the OCs explained less than 10% of model variability. The SSL from rookeries at Iony Island, Yamsky, and Srednego had a wide range of OC concentrations, were older when they were observed to reproduce, and had lower reproductive success. Because these locations did not have the highest OC concentrations compared to other rookeries, it is difficult to determine with certainty that OCs were having an effect, especially when other rookeries with comparable and greater concentrations were more reproductively successful. There did not appear to be an effect of OCs on reproductive parameters, but there may be implications for their offspring, where a large portion a female's OC load may be transferred to the pup during nursing (Lee et al. 1996) as well as exposure to the developing uterus during pregnancy (Wang et al. 2012, 2011). It would be expected that the first-born pup from a female with high OC concentrations would be at higher risk for negative health effects (Beckmen et al. 2003, 1999). Determining these effects would require hot-branding and collecting blood samples from mother-pup pairs.

Although our data indicate that OCs were not consistently having a negative effect on SSL, the concentrations of ∑PCB and ∑DDT measured in females from cohorts in 2001 – 2007 were above the proposed threshold (1 ppm) where negative biological effects could occur (Letcher et al. 2010). The concentrations for ∑PBDE were below but approaching this proposed threshold. The contaminants PCB, DDT, and HCB have been banned in most countries since the 1970's and 1960's, respectively, whereas several types of PBDE have been phased out of production since 2004 (Aguilar et al. 2002; Bard, 1999; Iwata et al. 1994, 1993). Despite restrictions and reduced usage of these chemicals the long-range transport, via atmospheric and oceanic advection, from countries that still use them resulting in deposition near SSL habitat (AMAP, 2004; Bard, 1999). The blood samples in our study are from SSL about three weeks old, which we used to determine correlations to survival, movement, and reproduction over a nine year period. Exposure of pups to OCs occurs at a vulnerable time in their development, but is an initial exposure level that will accumulate throughout their lives with periodic mobilization during fasting and pregnancy (Myers and Atkinson, 2012; Wang et al. 2011; Beckmen et al. 2003, 1999).

The effects of OCs may be manifested in different physiological functions that were not measured in this study, but nevertheless may be important on an individual scale if not the population scale. It is unlikely that the population decline and lack of recovery for the western population is attributable to any one cause (Atkinson et al. 2008), but the result of multiple stressors disproportionately affects population segments and cohorts. The insight gained by long-term monitoring of branded SSL in conjunction with biological data is an important area of continued research allowing for historical comparisons of SSL throughout their range in an effort to disentangle the causes of the concomitant decline and recovery.

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